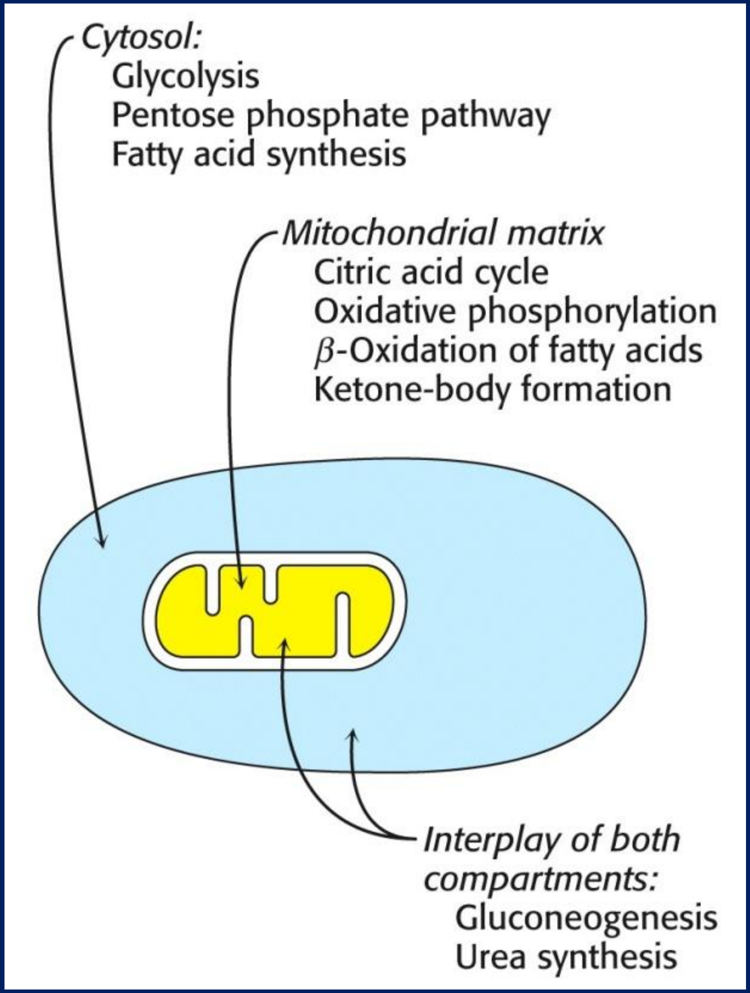
# Integration of metabolism

1. **Energetic integration** (NTPs (mainly ATP) are the universal currency of energy; main electron carriers in oxidative reactions are NADH and $FADH_2$, main electron donor in reductive biosynthesis is NADPH)
2. **Metabolic reactions integration in pathways** (few key metabolites, that are key junctions/nods of metabolic pathways; biomolecules are constructed from a small set of building blocks; enzymes’ activity is tightly regulated)

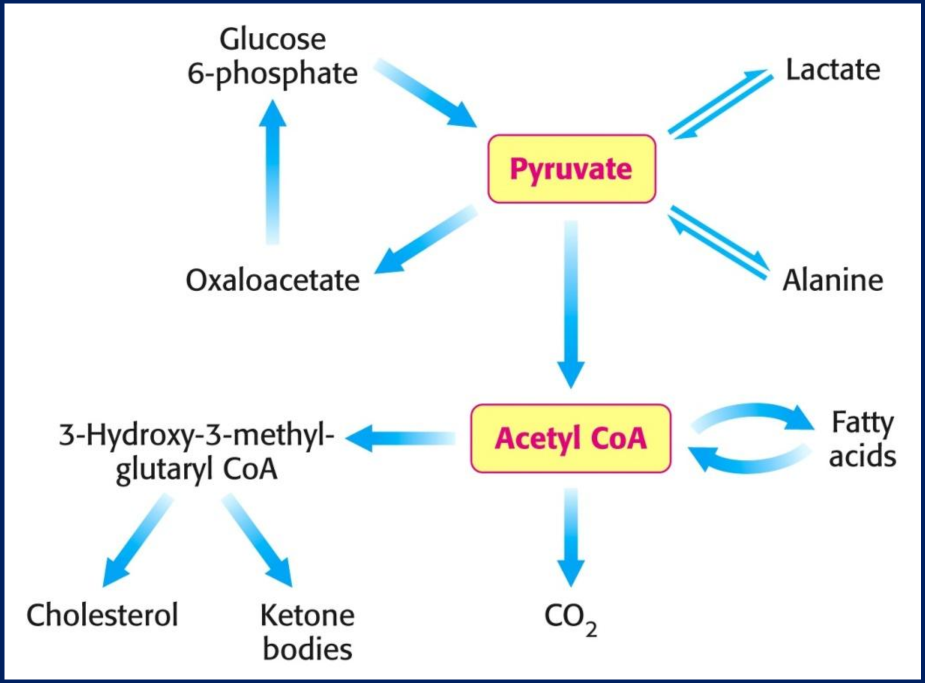
# Key Junctions between Pathways



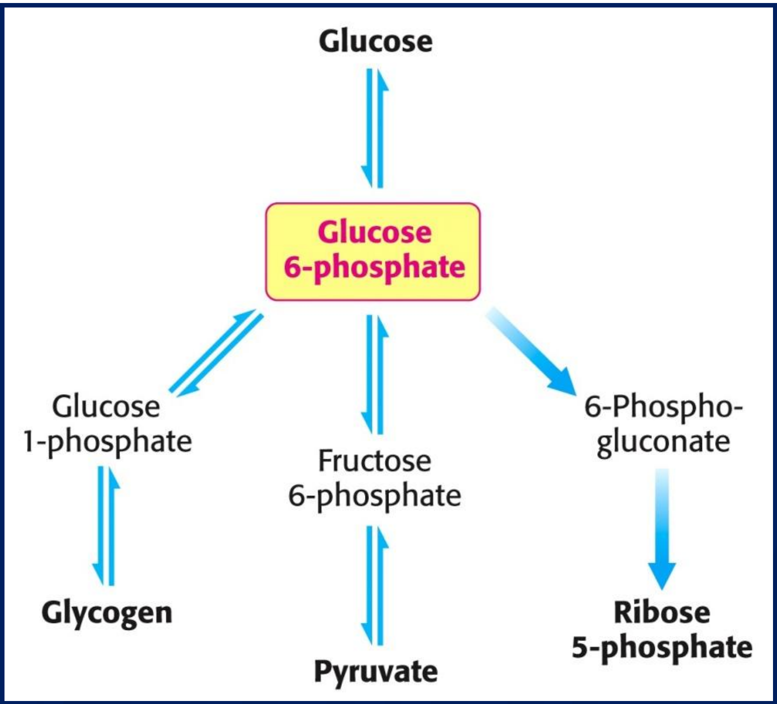
*Cytosol:*
Glycolysis
Pentose phosphate pathway
Fatty acid synthesis

*Mitochondrial matrix*
Citric acid cycle
Oxidative phosphorylation
$\beta$-Oxidation of fatty acids
Ketone-body formation

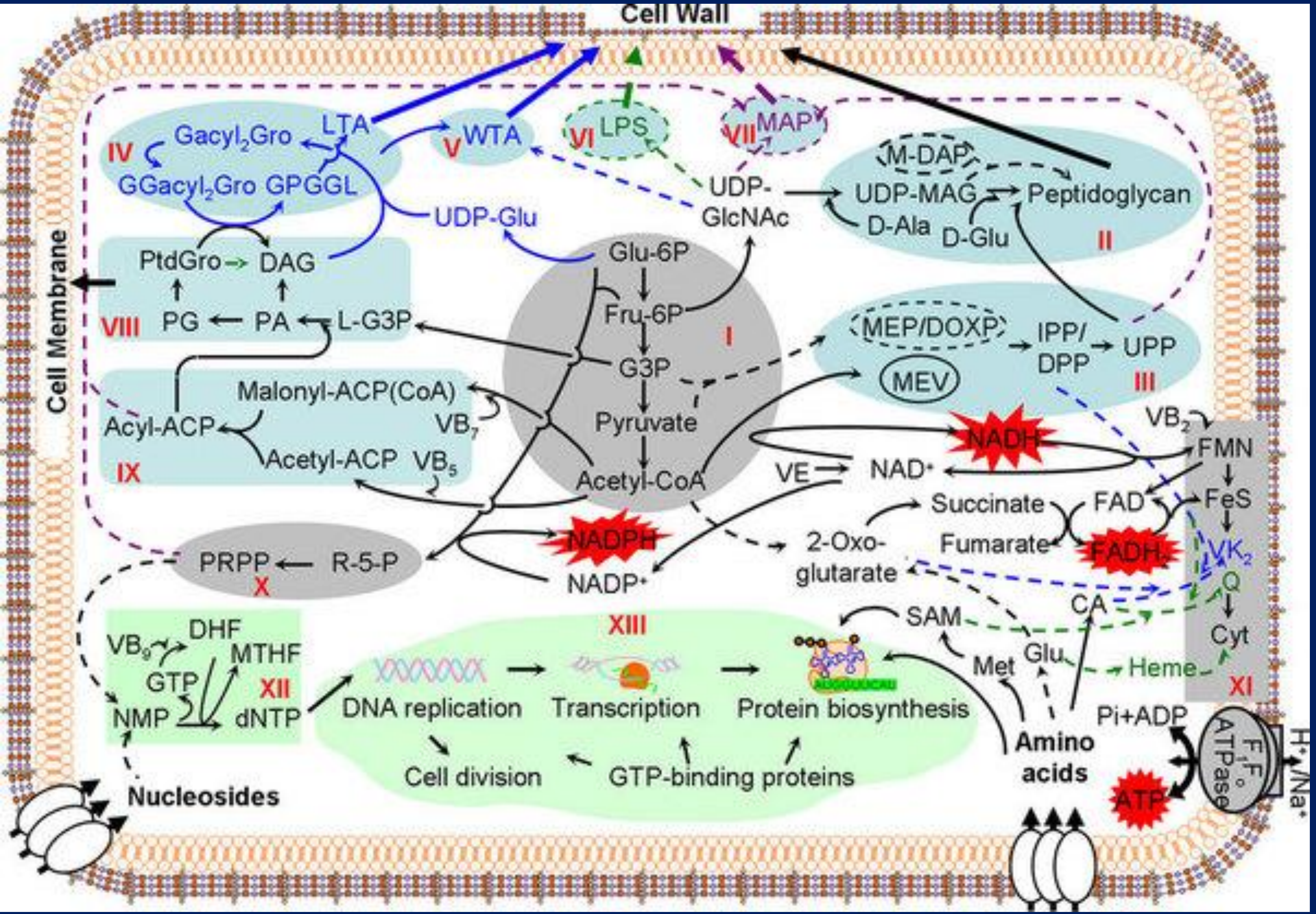
*Interplay of both compartments:*
Gluconeogenesis
Urea synthesis



Glucose 6-phosphate
Lactate
**Pyruvate**
Oxaloacetate
Alanine
3-Hydroxy-3-methyl-glutaryl CoA
**Acetyl CoA**
Fatty acids
Cholesterol
Ketone bodies
$CO_2$



**Glucose**
**Glucose 6-phosphate**
Glucose 1-phosphate
Fructose 6-phosphate
6-Phosphogluconate
**Glycogen**
**Pyruvate**
**Ribose 5-phosphate**


Cell Wall
Cell Membrane
IV
Gacyl$_2$Gro
GGacyl$_2$Gro
GPGGL
LTA
V
WTA
VI
LPS
VII
MAP
M-DAP
UDP-GlcNAc
UDP-MAG
D-Ala
D-Glu
Peptidoglycan
II
UDP-Glu
PtdGro
DAG
VIII
PG
PA
L-G3P
Glu-6P
Fru-6P
I
G3P
Pyruvate
Acetyl-CoA
MEP/DOXP
MEV
IPP/DPP
UPP
III
Malonyl-ACP(CoA)
VB$_7$
Acyl-ACP
Acetyl-ACP
VB$_5$
IX
NADH
VB$_2$
FMN
VE
NAD$^+$
FAD
FeS
Succinate
Fumarate
FADH$_2$
VK$_2$
Q
2-Oxo-glutarate
NADPH
NADP$^+$
PRPP
R-5-P
X
SAM
CA
Cyt
XIII
Met
Glu
Heme
XI
VB$_9$
DHF
MTHF
GTP
XII
NMP
dNTP
DNA replication
Transcription
Protein biosynthesis
Cell division
GTP-binding proteins
Amino acids
Pi+ADP
F$_1$F$_o$ ATPase
H$^+$/Na$^+$
ATP
Nucleosides

The three functions associated with essential pathways are indicated by color: pale blue, cell envelope;

- gray, energy production;
- light green, processing of genetic information.

Numbers are SSA#; red number, essential gene; black number, nonessential gene; solid arrow, an enzymatic reaction; dashed arrow, multistep pathway; blue dashed arrow, products involved in cell wall and membrane formation; block arrow, product from one pathway serving as input to another pathway; oval with bold arrow, transporter; slash, separating paralogs.

Ado, adenosine; Cyd, cytidine; DAG, 1,2-diacylglycerol; DHAP, dihydroxyacetone phosphate; DHF, dihydrofolate; L-G3P, sn-glycerol 3-phosphate; Guo, guanosine; LPA, lysophosphatidic acid; LTA, lipoteichoic acid; PA, phosphatidic acid; PGP, phosphatidylglycerophosphate; PPP, pentose phosphate pathway; TCS, two-component system; THF, tetrahydrofolate; Urd, uridine. In nucleotide biosynthesis, symbols are used to represent essential genes that participate in multistep reactions: SSA_1865 (green circle), SSA_0848 (yellow oval), SSA_2263, SSA_0771 (orange circles) and SSA_0768 and SSA_0770 (brown oval). Essential genes associated with ribosome and aminoacyl-tRNA biosynthesis are not indicated here, nor are SSA_0575, SSA_0800 and SSA_1903, which could not be categorized into these pathways.

# Integration and Regulation of Metabolic Functions

- Cells synthesize or degrade channel and transport proteins
- Cells often synthesize enzymes needed to catabolize a particular substrate only when that substrate is available
- If two energy sources are available, cells catabolize the more energy efficient of the two first.
- Cells synthesize the metabolites they need, but typically cease synthesis if metabolite is available

# Integration and Regulation of Metabolic Functions

- Eukaryotic cells keep metabolic processes from interfering with each other by isolating particular enzyme within membrane-bounded organelles
- Cells use allosteric sites on enzymes to control the activity of enzymes
- Feedback inhibition slows or stops anabolic pathways when product is in abundance
- Cells regulate amphibolic pathways that use the same substrate by requiring different coenzymes for each

# Regulation of enzymatic reactions

- Regulation of enzyme *structure* as protein
- Regulation of enzyme *quantity* by change of gene expression or rate of degradation
- Regulation *of substrates* and/or *cofactors/coenzymes* concentration and access by compartmentation and redistribution (transport, competing between enzymes, binding),
- Regulation of *pH and physical conditions* for reaction
- Action of *inhibitors or activators*
- Existence of *isoenzymes*

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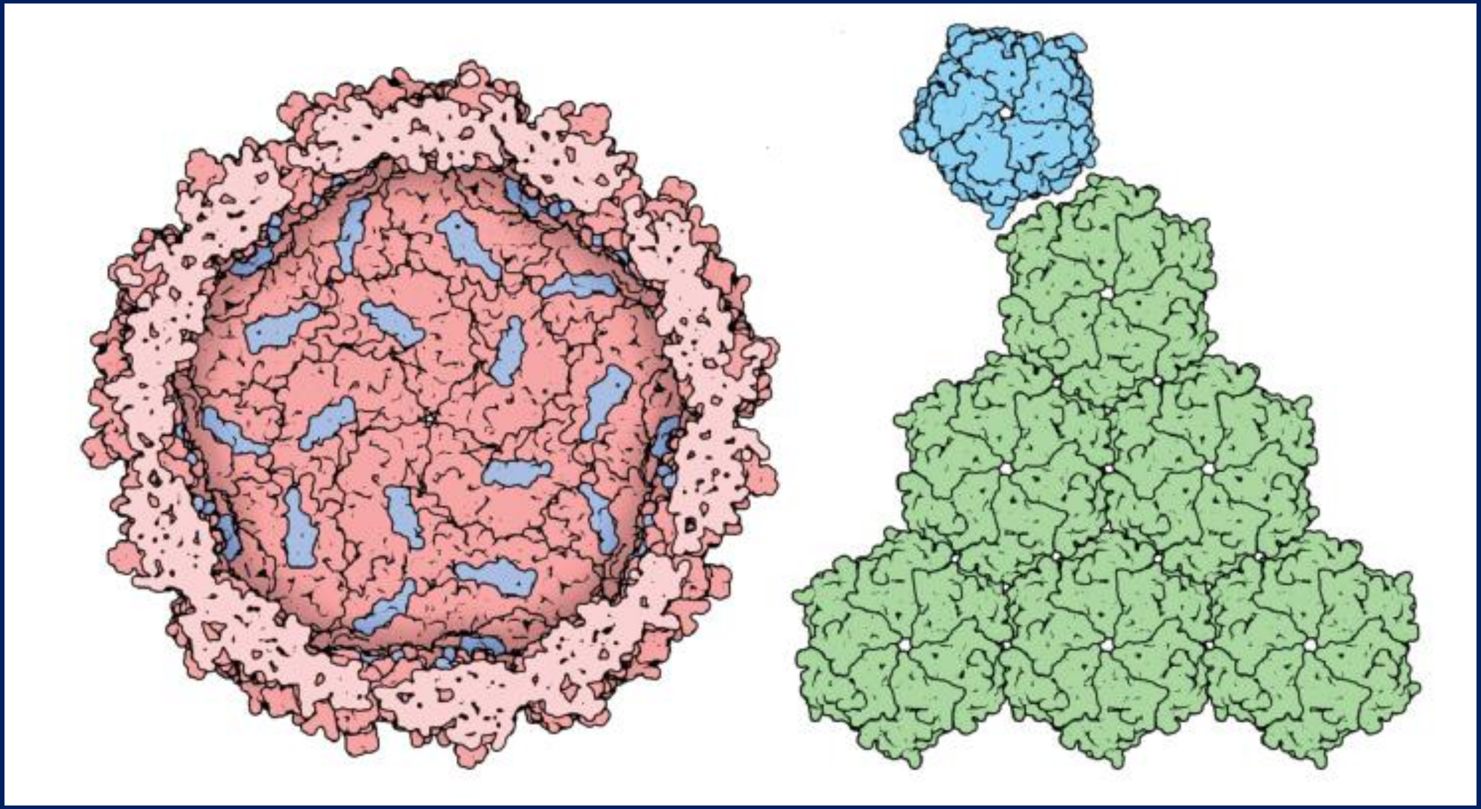
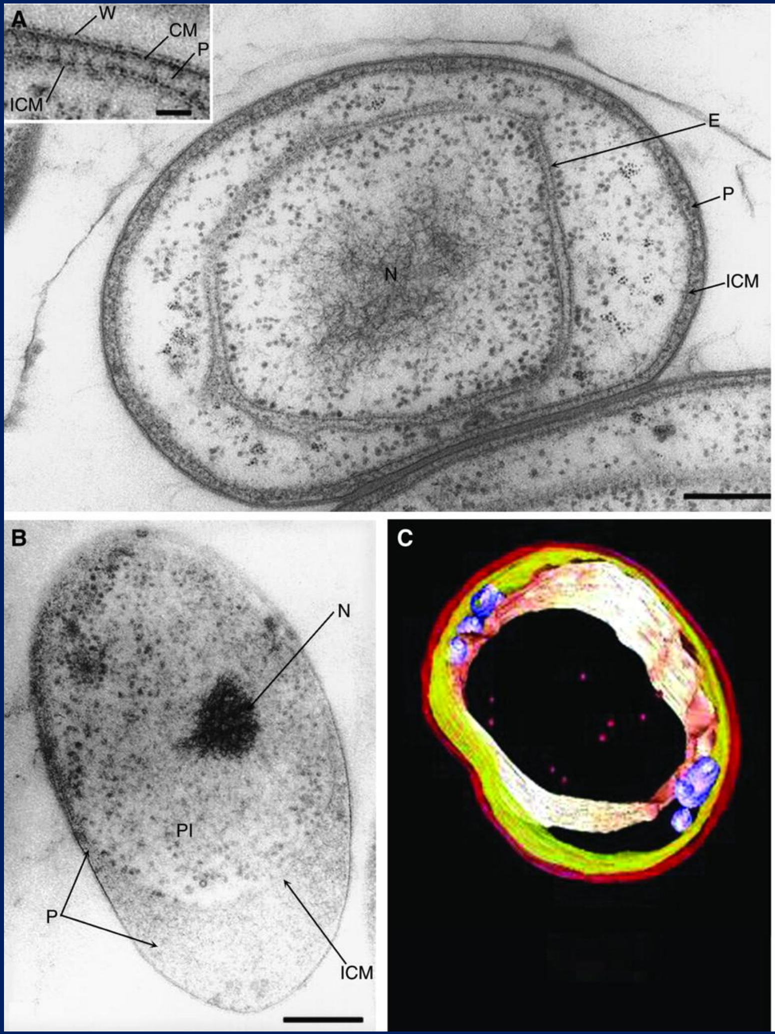
# Bacterial microcompartments



# The nucleus-like organelle of Gemmata obscuriglobus is shown in (A).



**Murat D et al. Cold Spring Harb Perspect Biol 2010;2:a000422**

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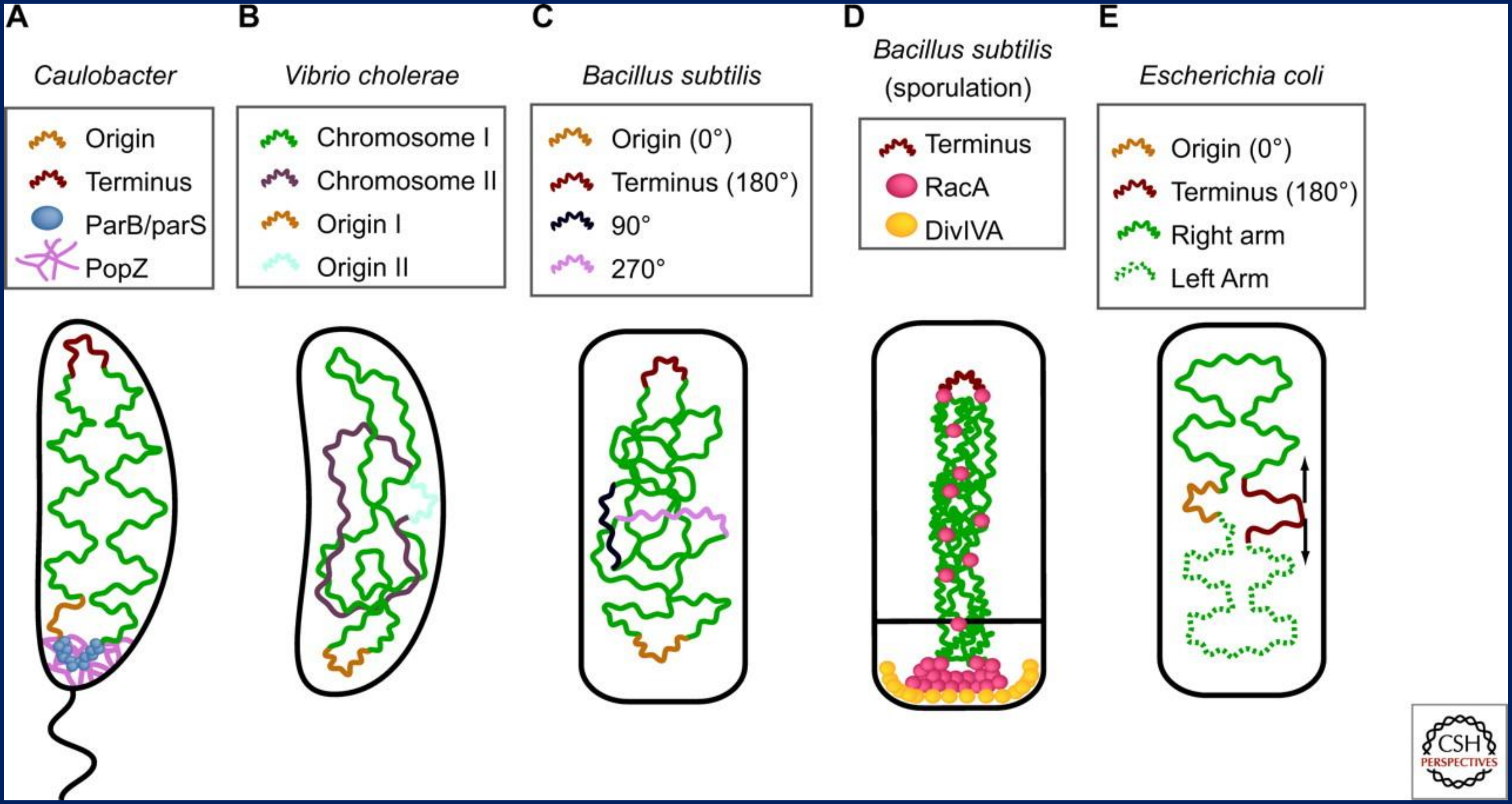
# Chromosome organization in model bacteria.



Toro E , and Shapiro L Cold Spring Harb Perspect Biol 2010;2:a000349

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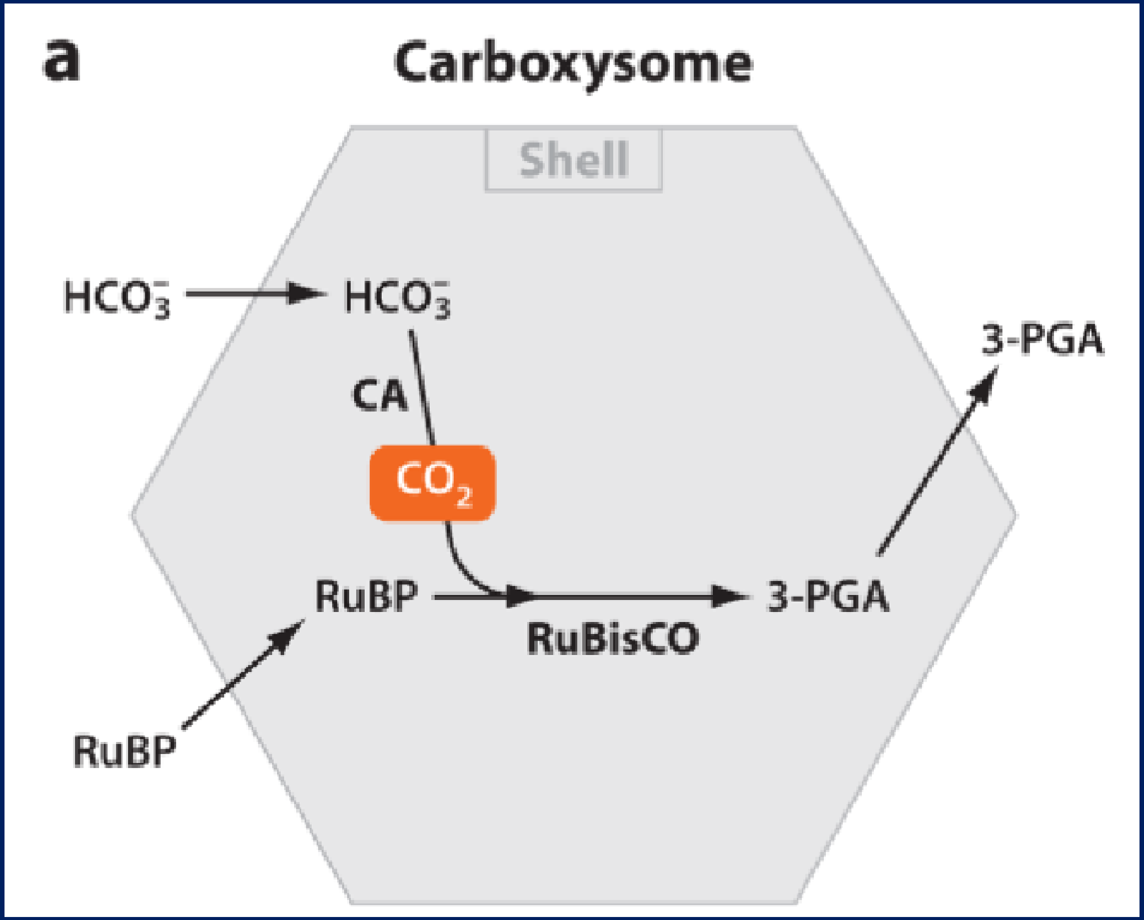
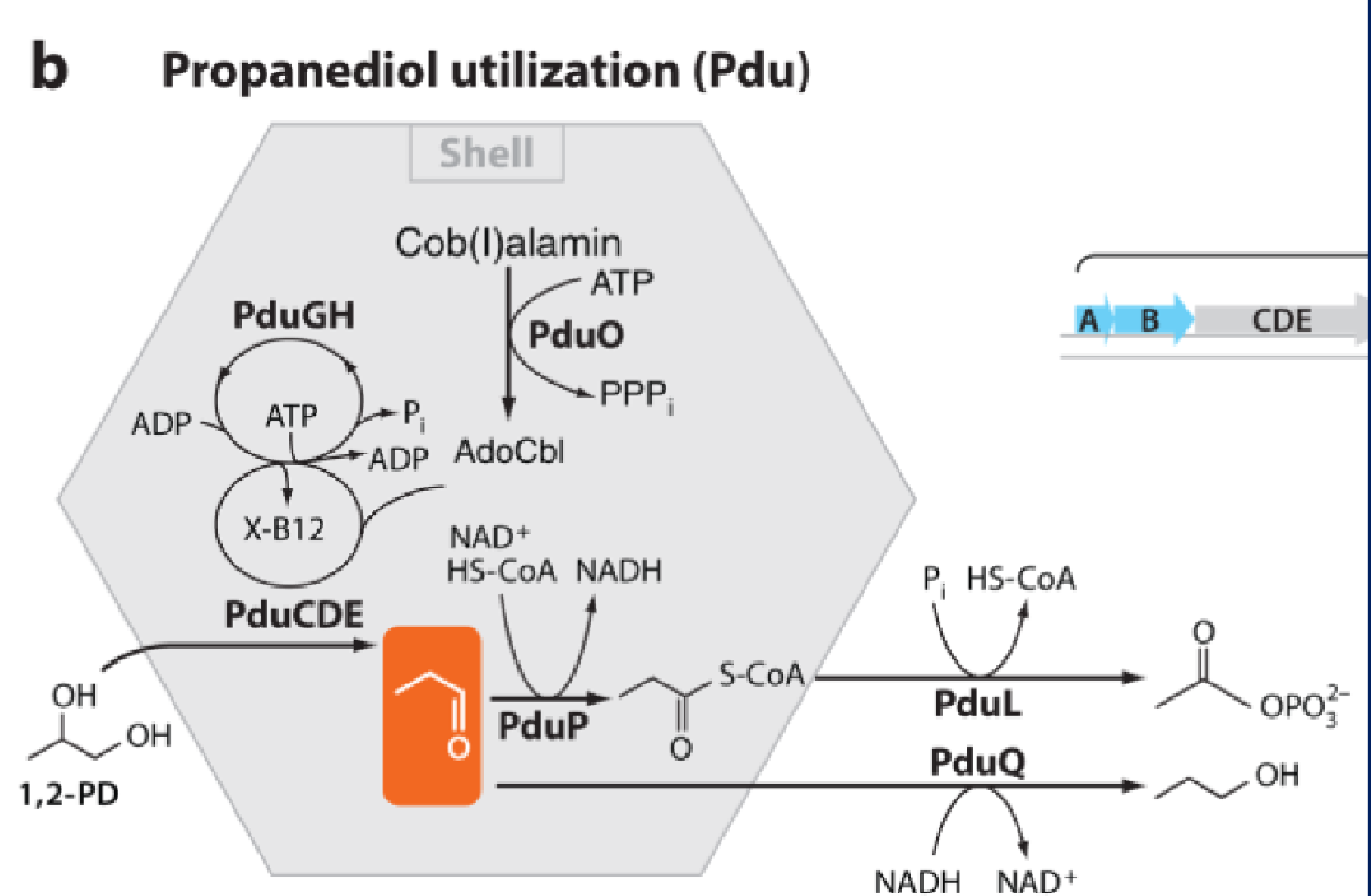
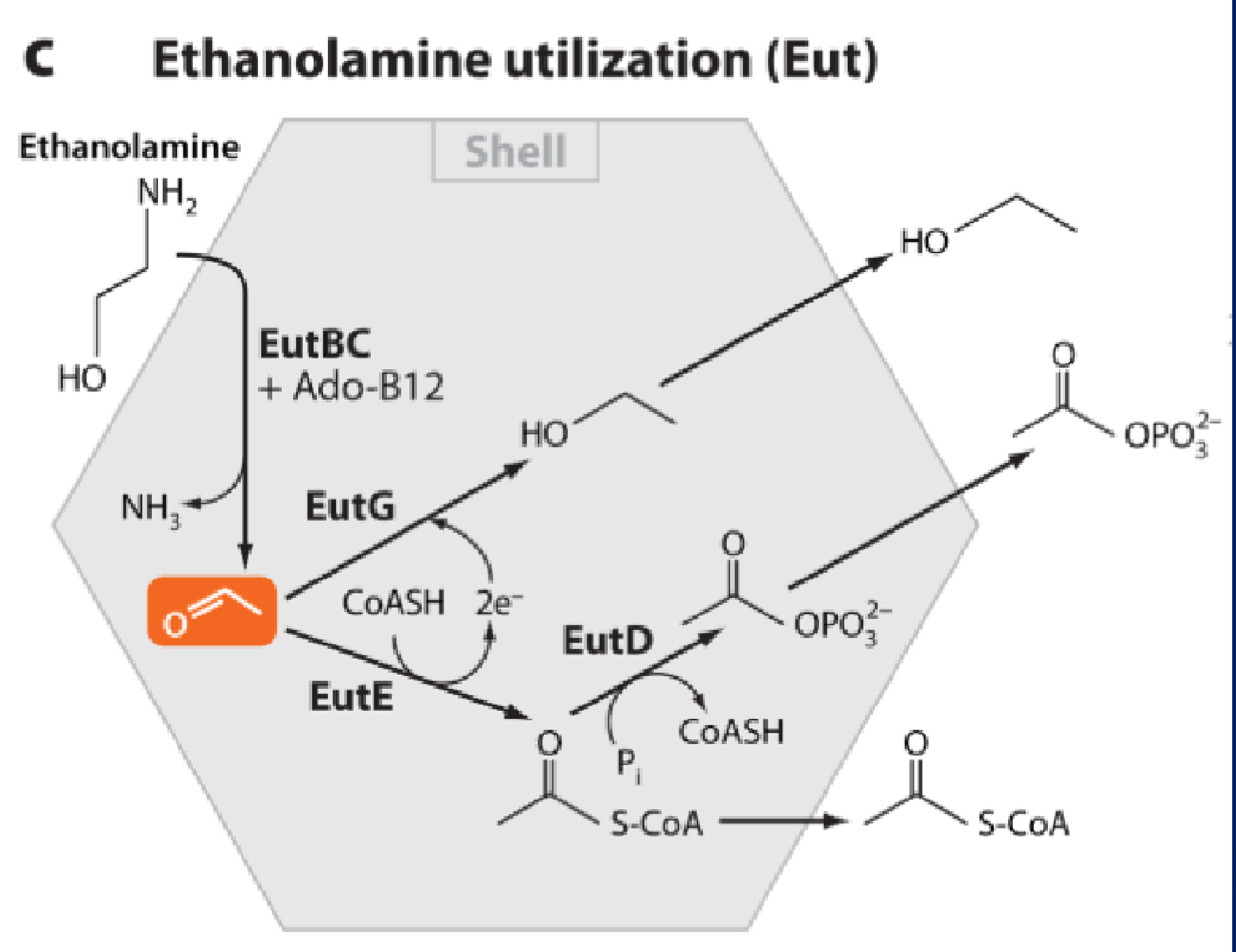
# Metabolic specificity of microcompartments



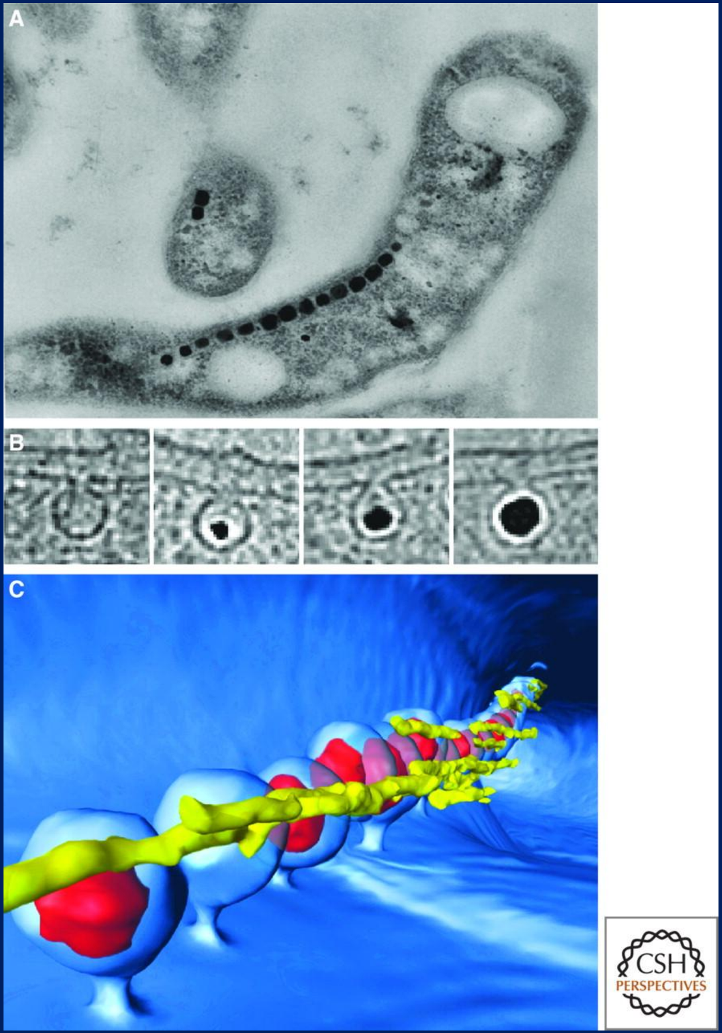


b
Propanediol utilization (Pdu)
Shell
Cob(I)alamin
ATP
PduO
PPP$_i$
AdoCbl
PduGH
ADP
ATP
P$_i$
ADP
X-B12
PduCDE
NAD+
HS-CoA
NADH
PduP
S-CoA
P$_i$
HS-CoA
PduL
O
OPO$_3^{2-}$
PduQ
OH
NADH
NAD+
OH
OH
1,2-PD
O
O
A
B
CDE



**c** Ethanolamine utilization (Eut)

# Magnetosomes can be easily visualized with various forms of electron microscopy.



Murat D et al. Cold Spring Harb Perspect Biol 2010;2:a000422

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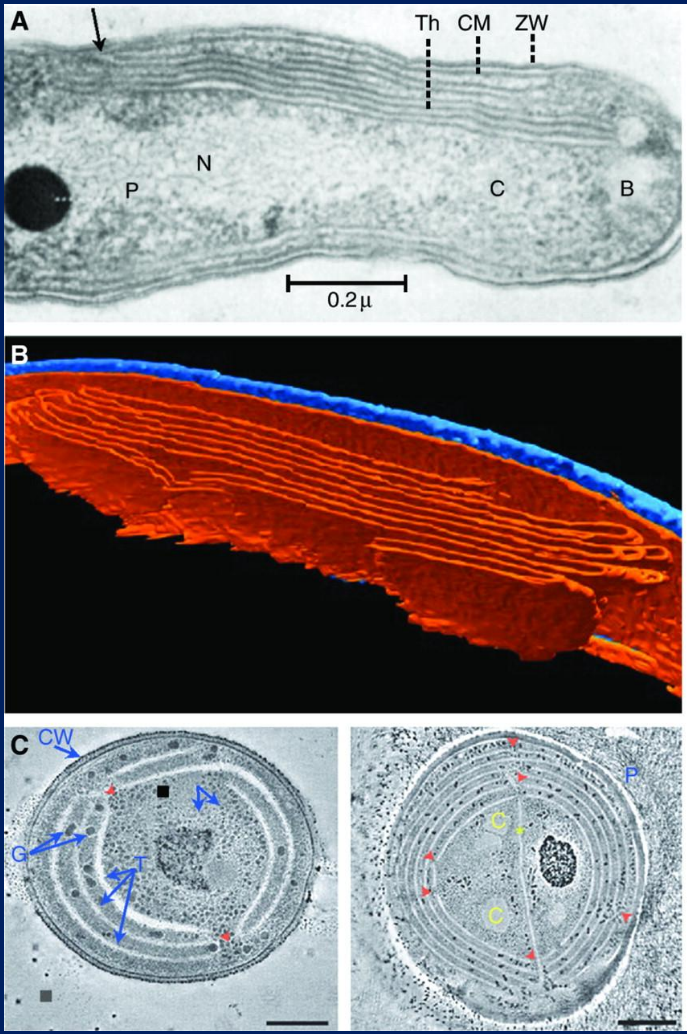
# Photosynthetic membranes were the first of bacterial organelles to be imaged with electron microscopy.



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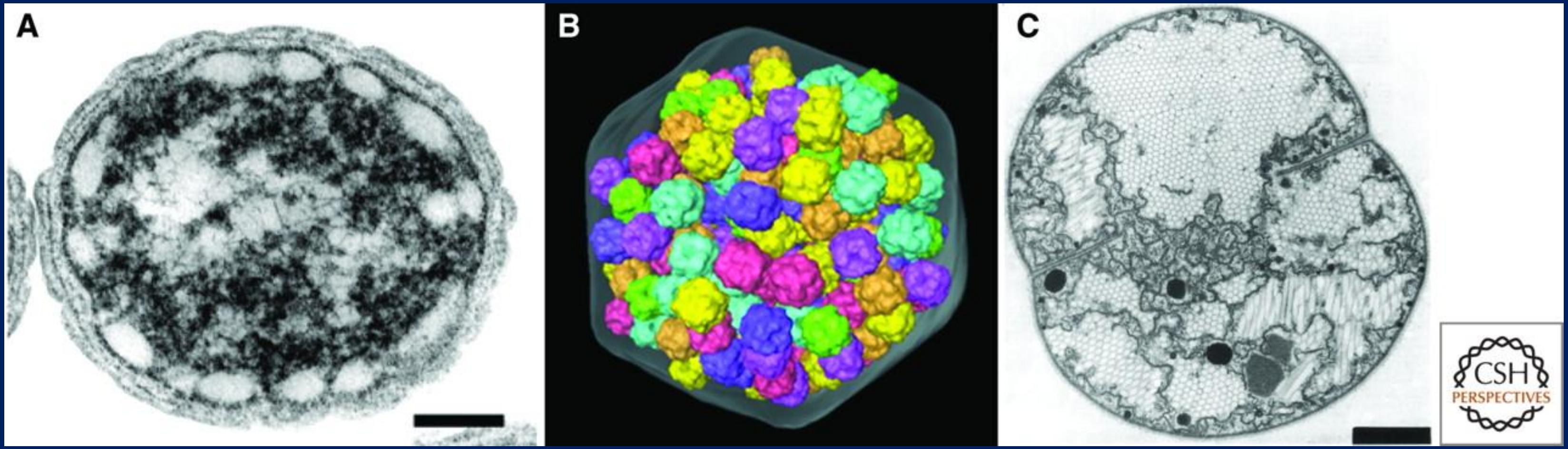
# Chlorosomes of Chlorobium tepidum appear as flattened ovals arranged around the cell periphery (A).



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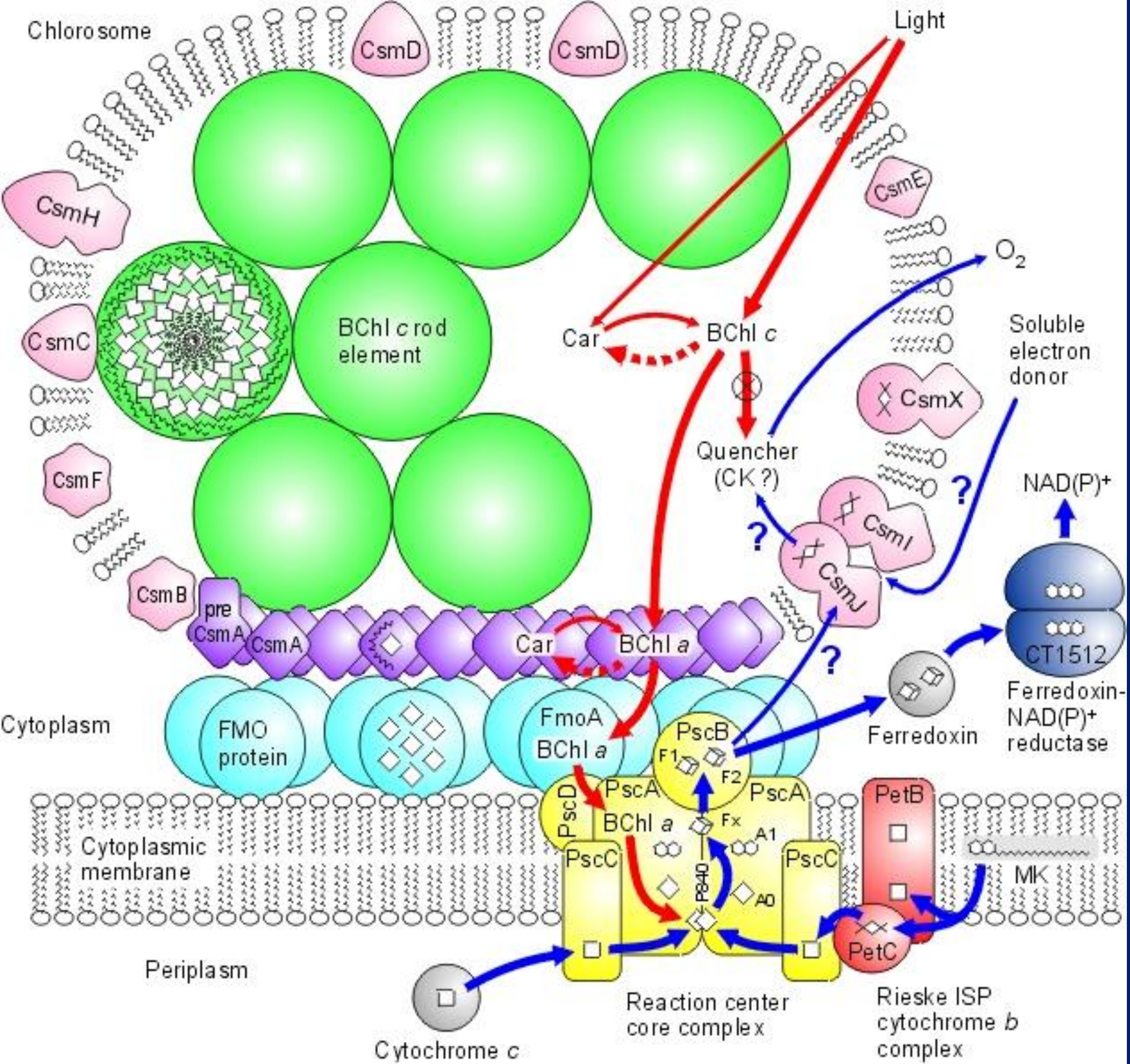


The major light-harvesting antenna structure in photosynthetic green bacteria is the **chlorosome**.

The chlorosome is of large size and its main antenna pigment, BChl c, is not organized on a protein scaffold but is "self-organizing" by molecular aggregation

http://www.bio.ku.dk/nuf/research/chlorosome.htm

# Hierarchical assembly of typical bacterial microcompartment

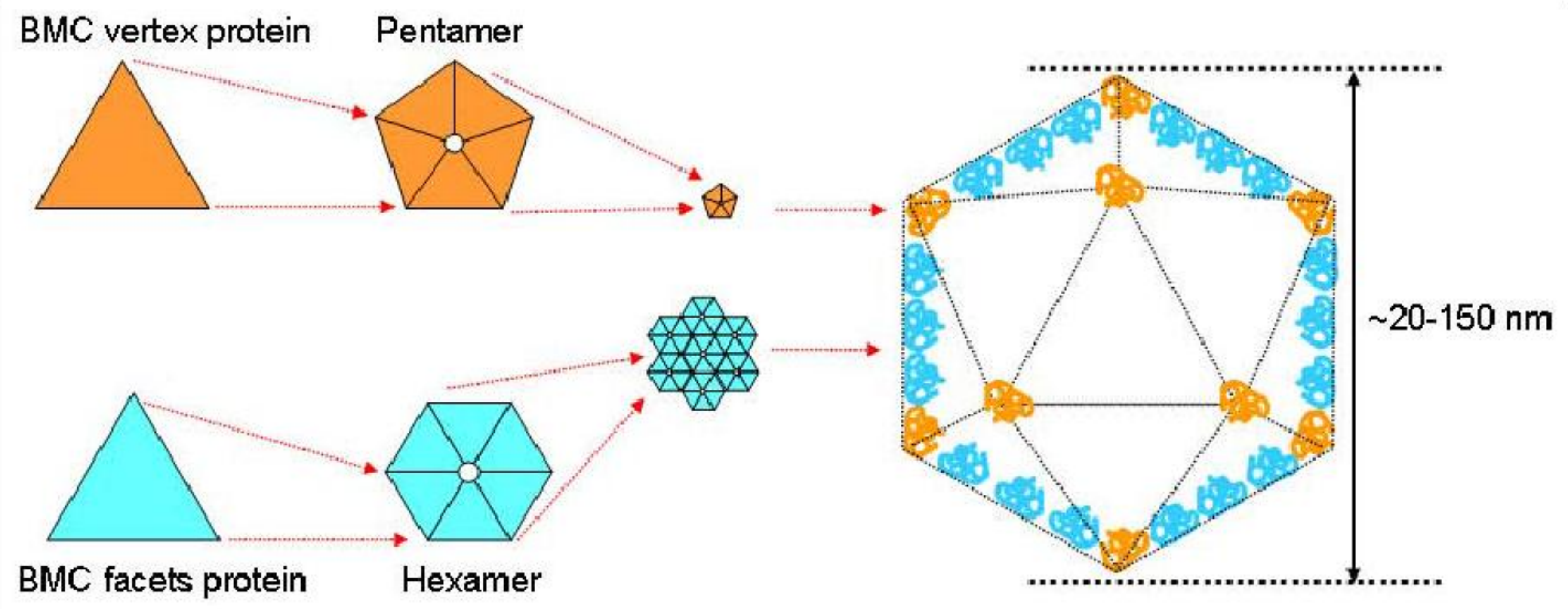
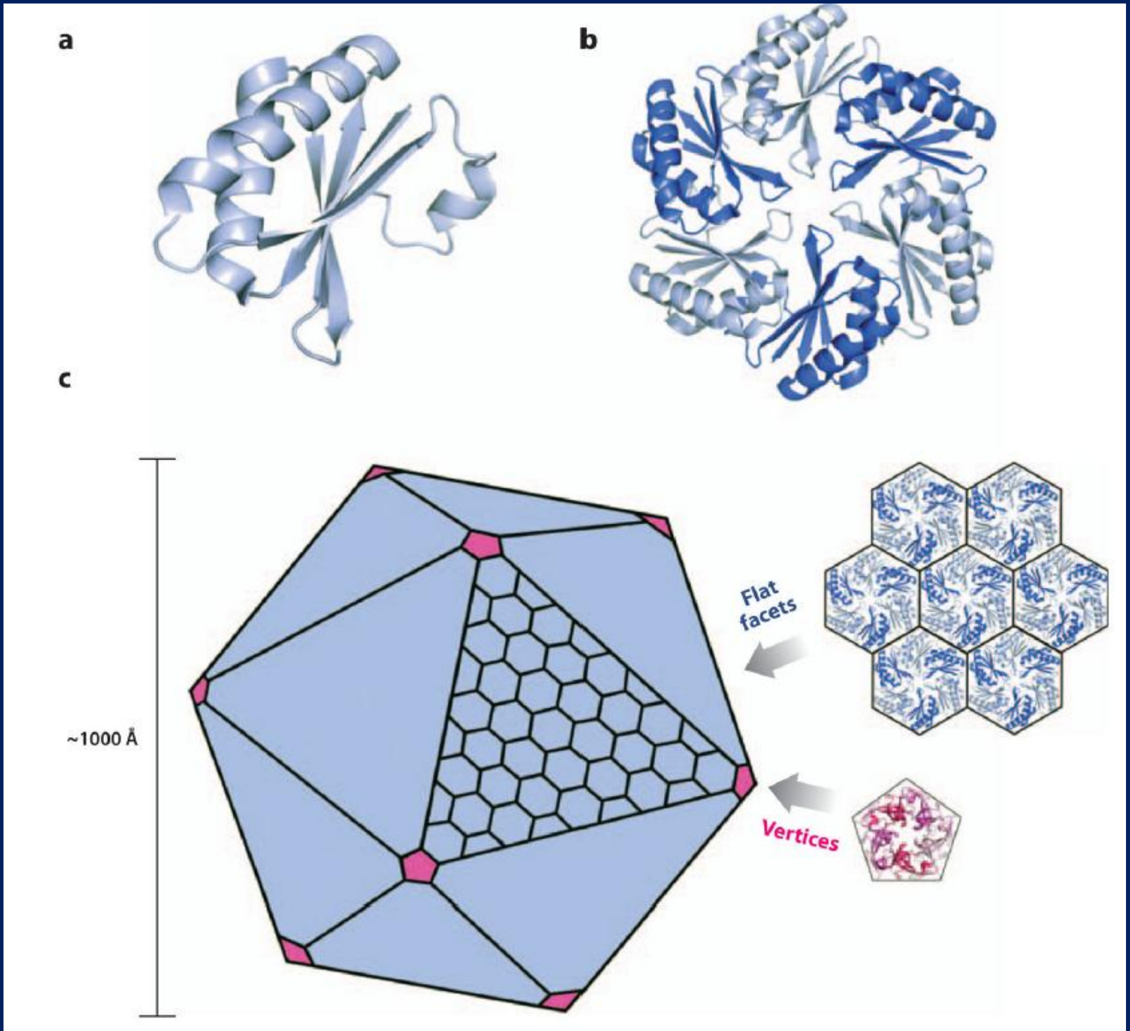


**Figure 1 Model for the hierarchical assembly of a typical bacterial microcompartment**. The facets of these icosahedral nano-cages are made of one type of protein (in light blue) that further self-assemble to give hexamers. On the other hand, the vertices are formed by pentamers resulting of the self-assembly of a different protein (in orange). Pores allowing traficking of molecules are located at the center of each hexamer and pentamer. Sizes can range from 20-25 nm for the encapsulin shell to 100-150 nm for carboxysomes.


a
b
c
~1000 Å
Flat facets
Vertices

# Integration of metabolism

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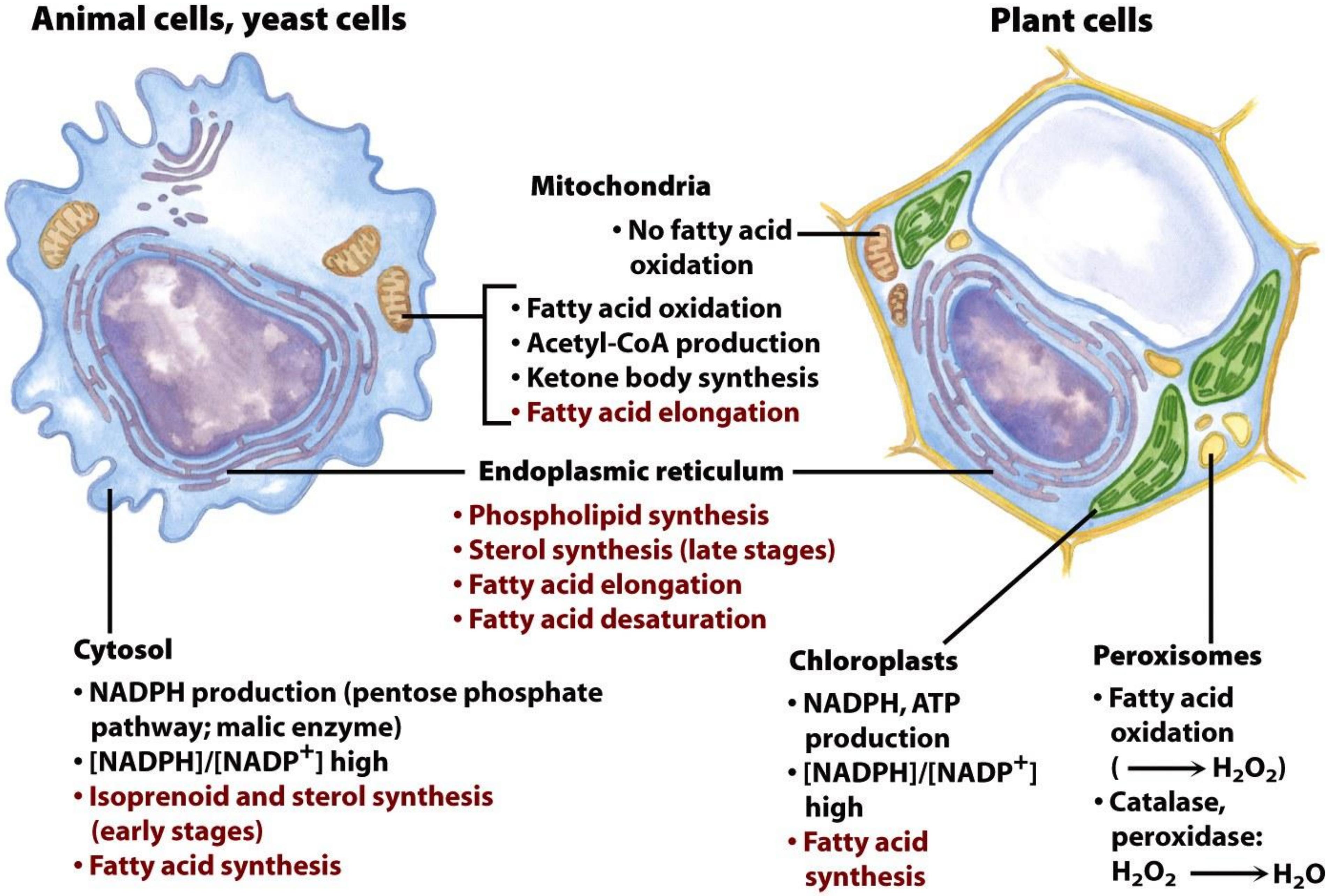
# Eukaryotic compartments and cell structure



**Animal cells, yeast cells**

**Plant cells**

**Mitochondria**

- No fatty acid oxidation

- Fatty acid oxidation
- Acetyl-CoA production
- Ketone body synthesis
- Fatty acid elongation

**Endoplasmic reticulum**

- Phospholipid synthesis
- Sterol synthesis (late stages)
- Fatty acid elongation
- Fatty acid desaturation

**Cytosol**

- NADPH production (pentose phosphate pathway; malic enzyme)
- [NADPH]/[NADP$^+$] high
- Isoprenoid and sterol synthesis (early stages)
- Fatty acid synthesis

**Chloroplasts**

- NADPH, ATP production
- [NADPH]/[NADP$^+$] high
- Fatty acid synthesis

**Peroxisomes**

- Fatty acid oxidation ($\longrightarrow H_2O_2$)
- Catalase, peroxidase: $H_2O_2 \longrightarrow H_2O$

**Figure 21-8**

*Lehninger Principles of Biochemistry, Fifth Edition*

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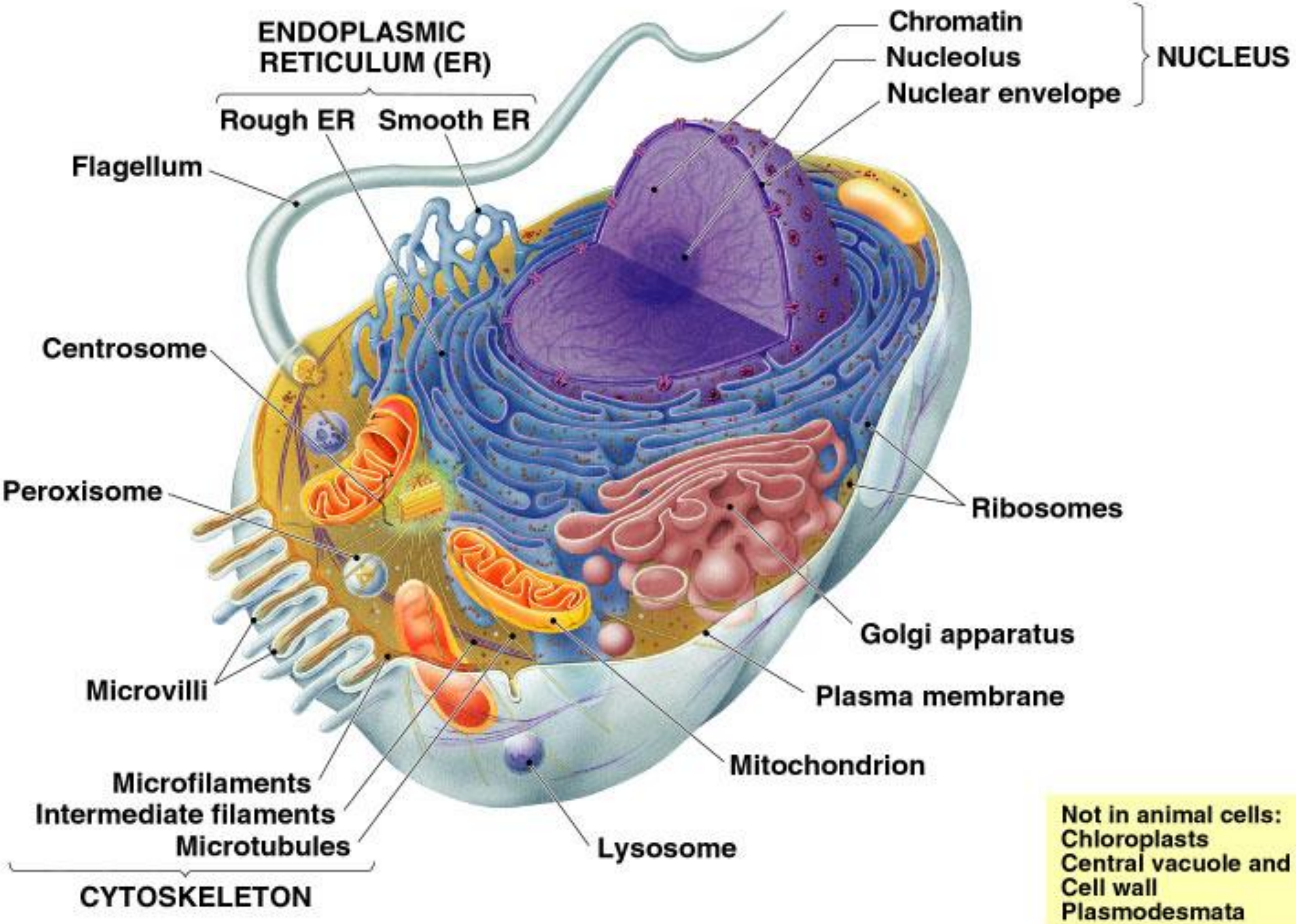

**Not in animal cells:**
**Chloroplasts**
**Central vacuole and tonoplast**
**Cell wall**
**Plasmodesmata**

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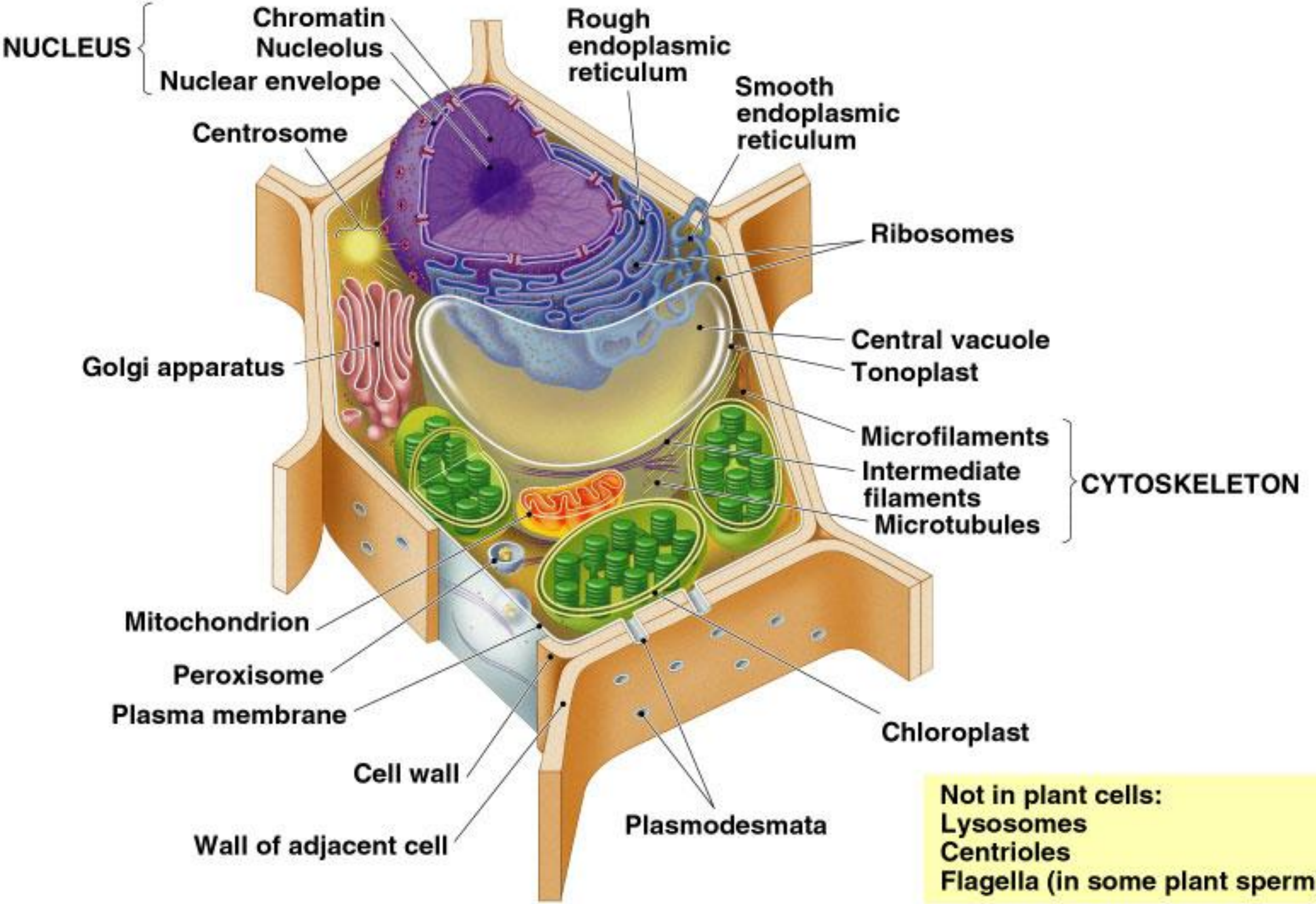

**Not in plant cells:**
**Lysosomes**
**Centrioles**
**Flagella (in some plant sperm)**

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# Участие белков в организации хроматина в ядре

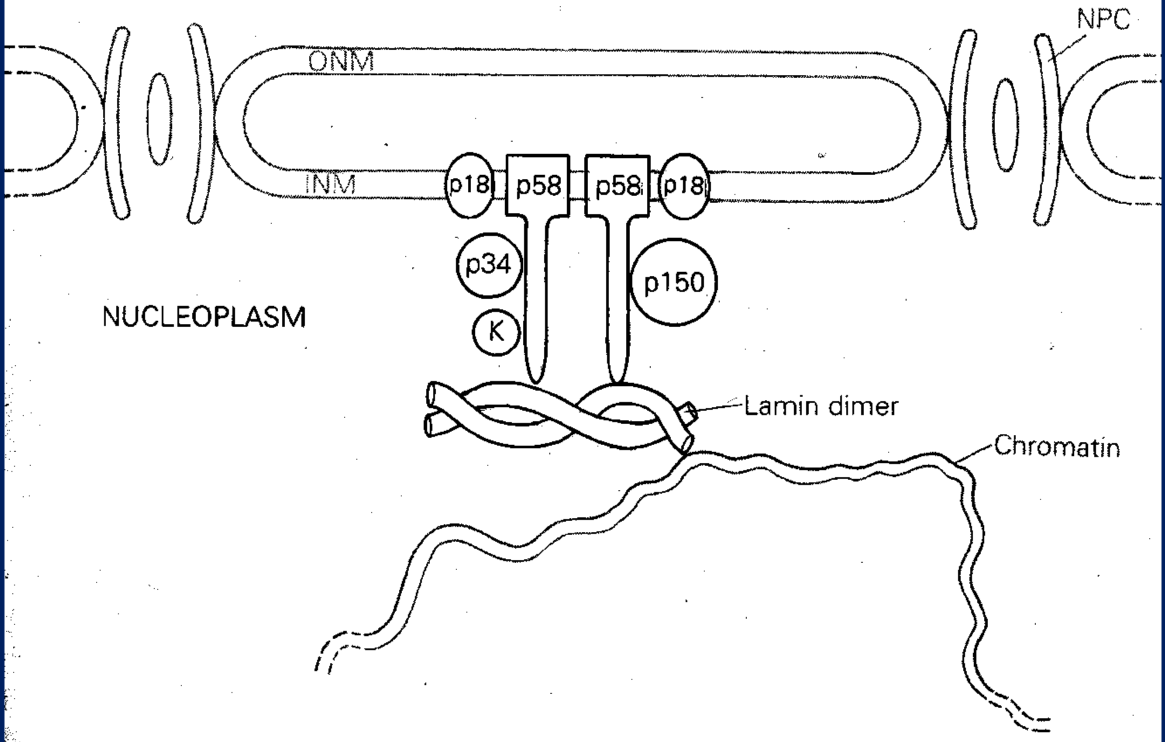
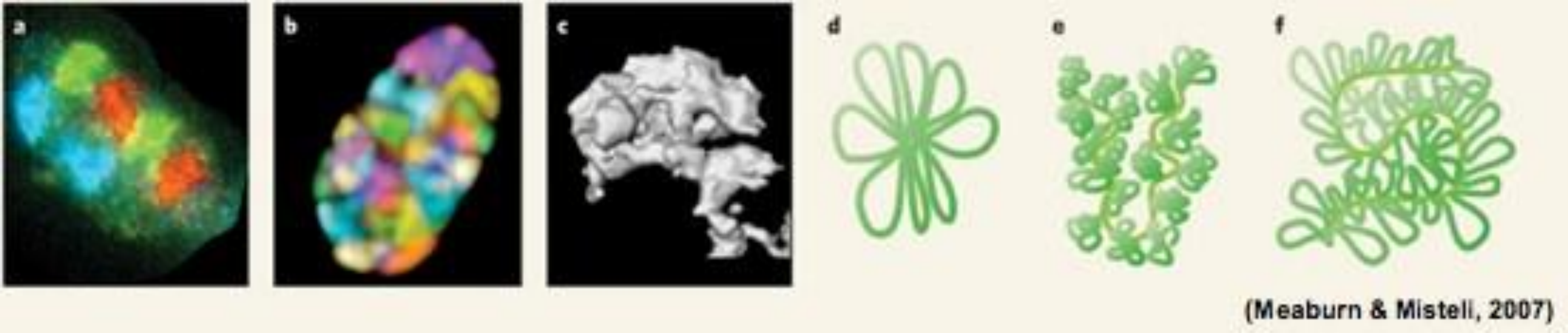


***Fig. 4.33*** The p58 complex of the inner nuclear membrane. See text for details. Redrawn from Georgatos, S.D., Meier, J. and Simos, G. (1994) Lamins and lamin-associated proteins. *Current Opinion in Cell Biology*, **6**, 347–353.

# Chromosome Territories



(Meaburn & Misteli, 2007)

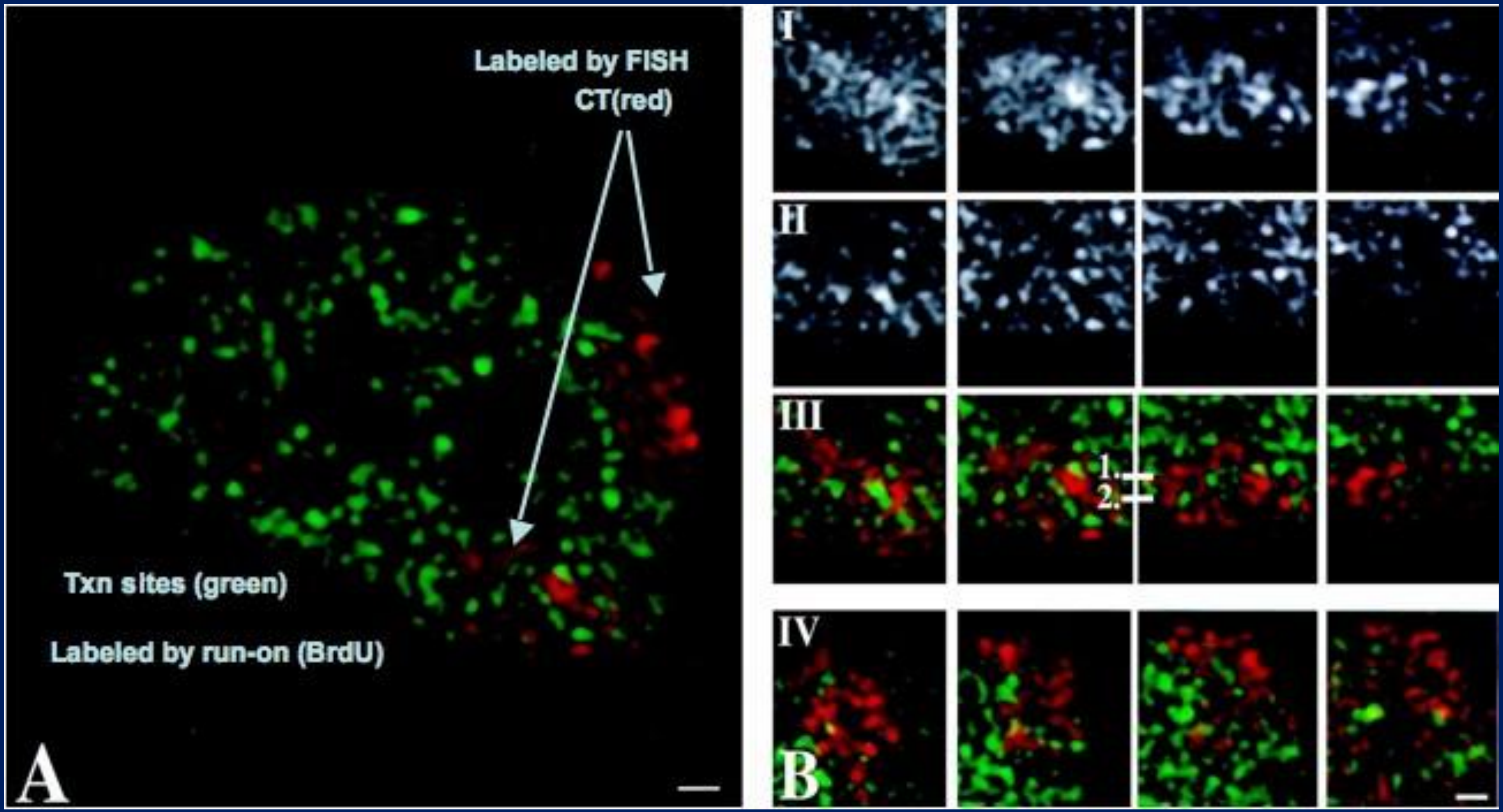
- Individual chromosomes are organized into chromosome territories (CTs)

- Chromatins are dynamic - interactions with nuclear architecture

- Correlation between CT structure and function (active vs. inactive X chromosome)

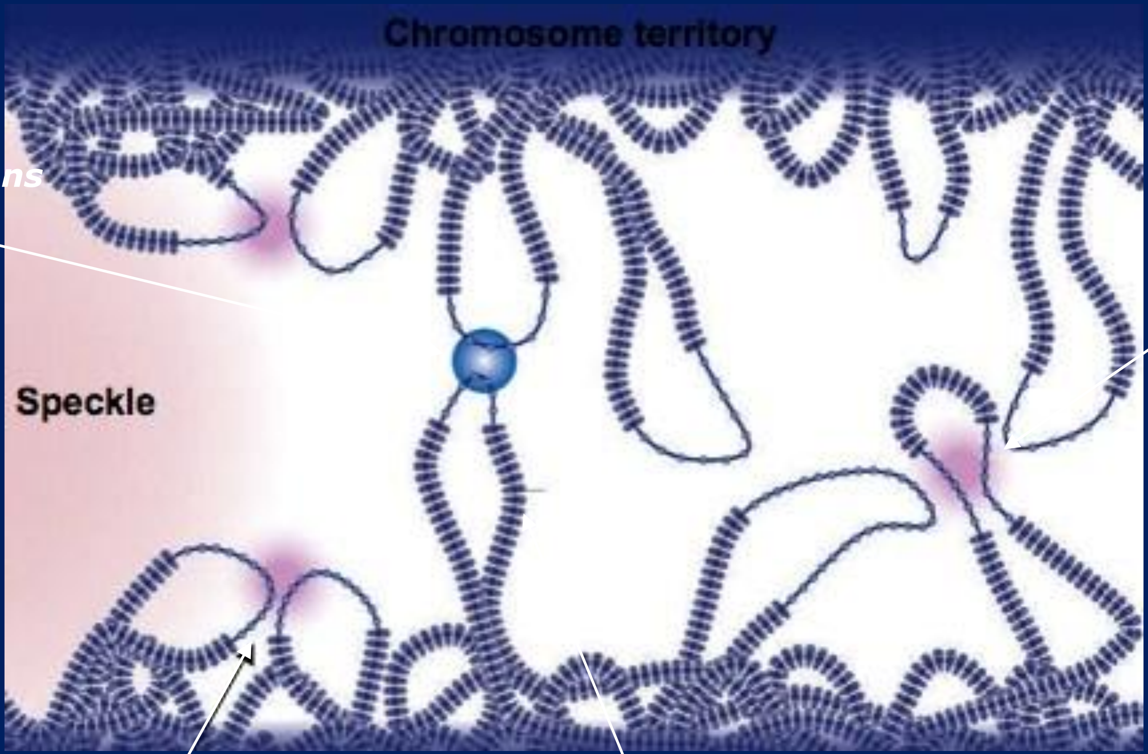
Purpose: to facilitate/regulate gene expression

# Correlation between chromosome territories & gene activity



Distribution of transcription sites in relations to CTs

# Colocalization of genes in the nucleus for expression or coregulation



***Cis*-interaction/*trans* interaction**

***Cis* and *trans* co-association**

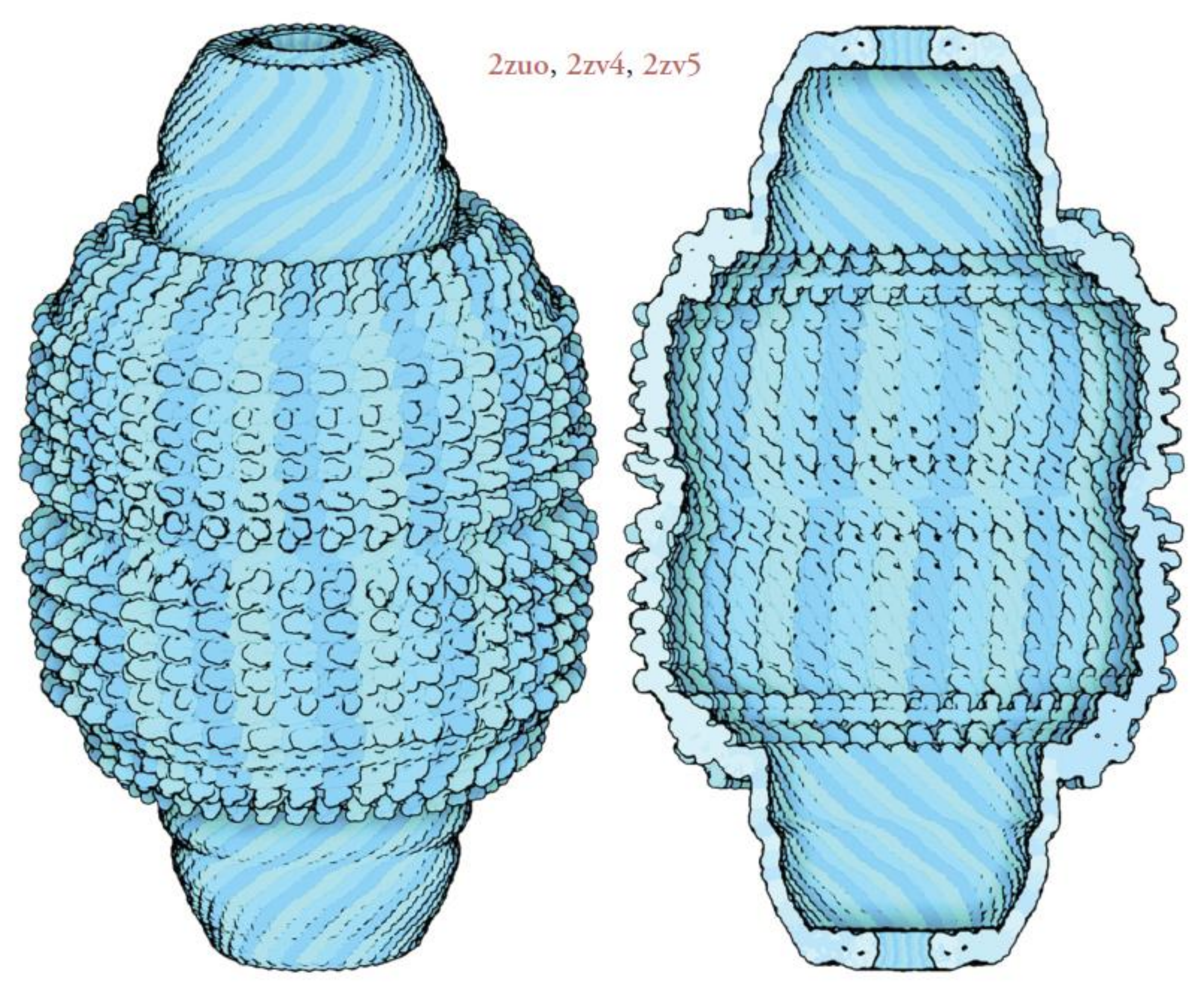
**Transcription factory**

**Chromatin loop**

**(Fraser & Bickmore, 2007)**

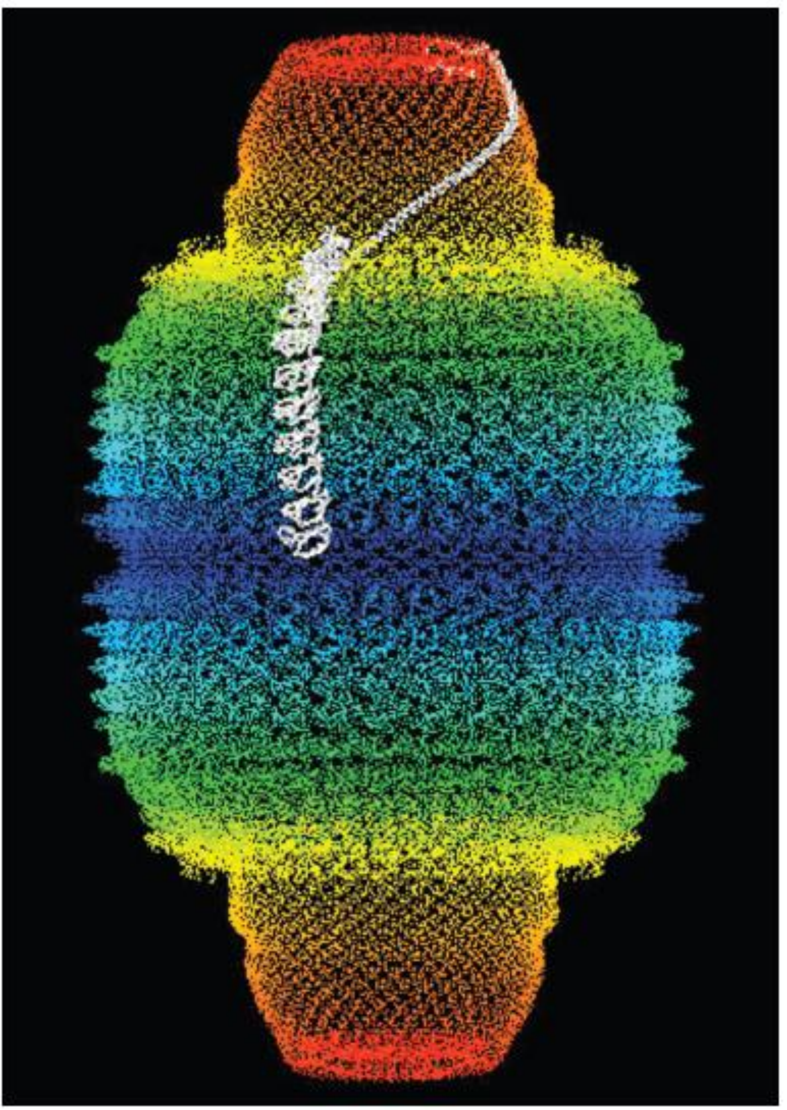
Correlation between chromosome location and gene expression

# Vaults



2zuo, 2zv4, 2zv5

The vault is a huge symmetrical structure composed of 78 identical chains. In this picture, one of the chains is colored white and the others are colored from red to blue from one end of the chain to the other. Part of the protein folds into a string of small domains that together assemble into the wide body at the center. The rest of the protein forms a long alpha helix that assembles into the narrow cap at the ends. The vault is so large that the coordinates have been split into three PDB entries (2zuo, 2zv4, and 2zv5) so watch your step when you go to explore this structure yourself!



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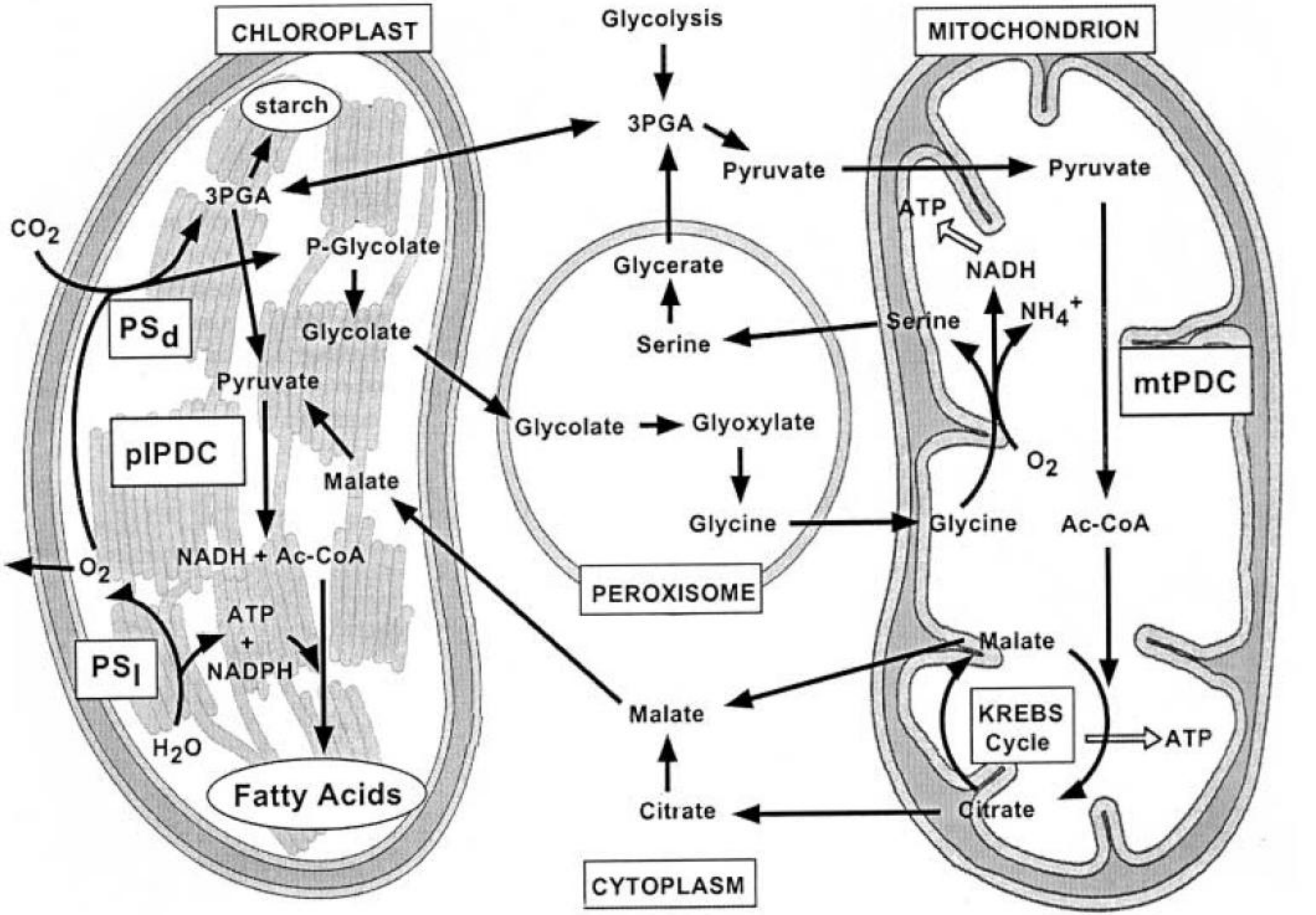

**Fig. 1. Compartmentalization of metabolism in plant cells.** $PS_l$, the light reactions of photosynthesis; $PS_d$, the dark reactions of photosynthesis.

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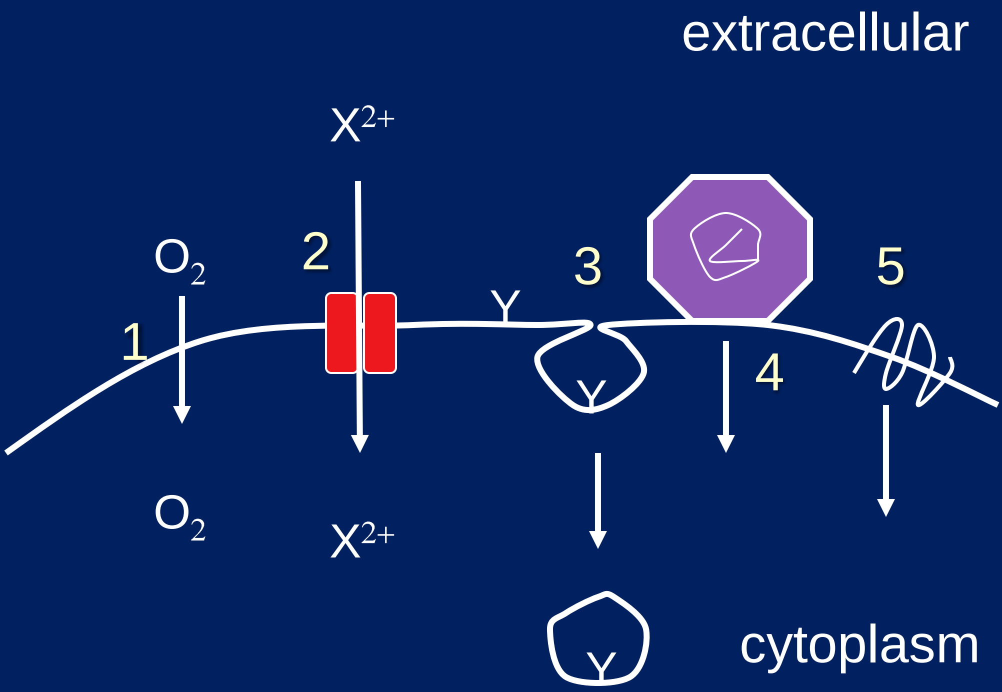
   metabolic reactions are compartmented; biosynthesis and catabolism are usually separated in space;
   **compartmentation is supported by regulated molecules' transport, sorting and assembly**

# Getting molecules into cells: crossing the plasma membrane

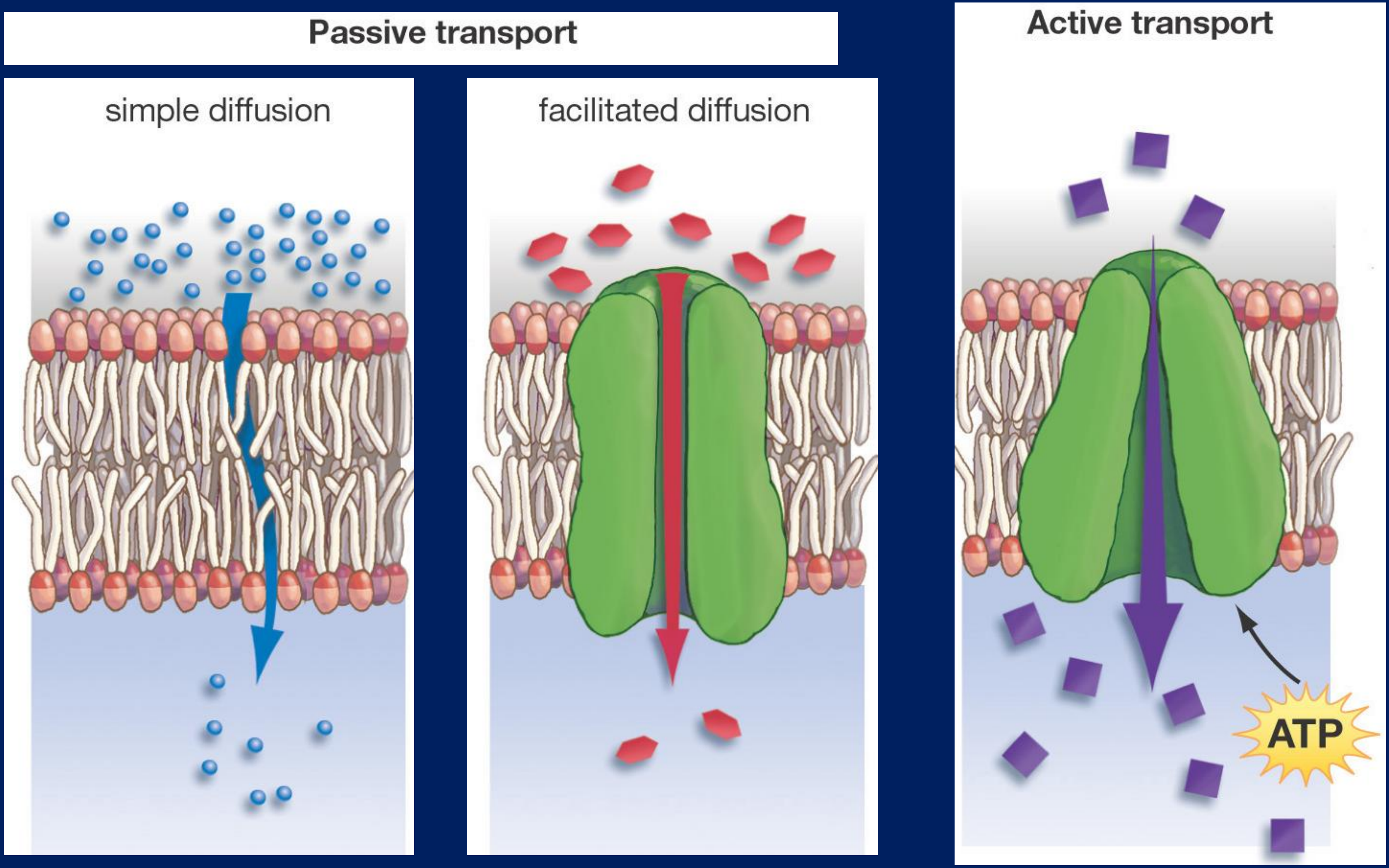
1. Diffusion across the plasma membrane /*water, gases, small molecules*
2. Protein-mediated transport /ion channels, transporters
3. Formation and internalization of membrane-limited vesicles/

   Endocytosis
   Pinocytosis
   Phagocytosis

4. Membrane fusion /viruses
5. Pore formation /toxins



# Three Forms of Transport Across the Membrane



Example: Oxygen or water diffusing into a cell and carbon dioxide diffusing out.

Examples: Glucose or amino acids moving from blood into a cell.

Examples: Pumping $Na^+$ (sodium ions) out and $K^+$ (potassium ions) in against strong concentration gradients.

# Gated Channels

- Control Transmembrane Potential
- Voltage Gated
- Ligand Gated

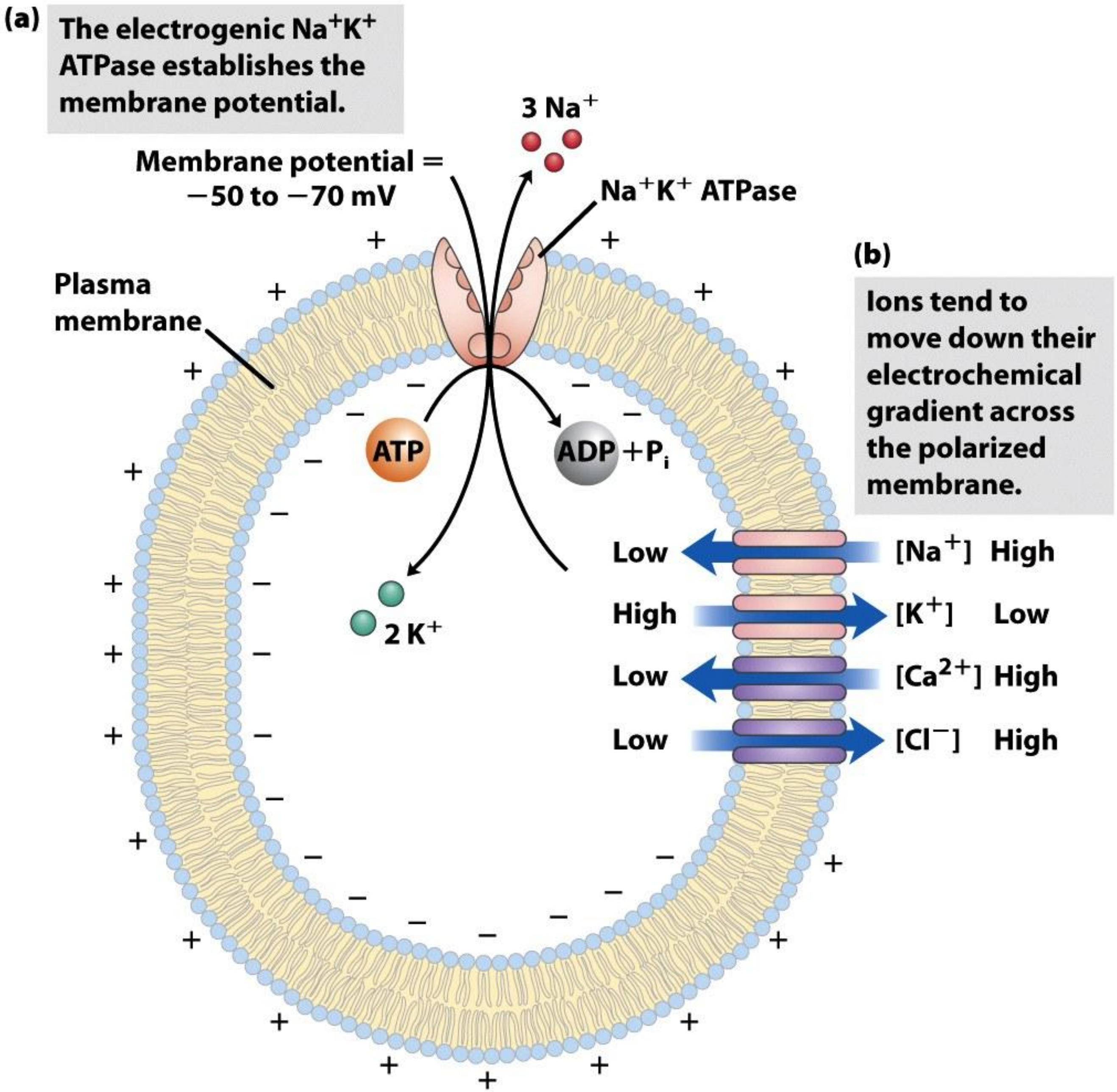

(a)
The electrogenic Na⁺K⁺ ATPase establishes the membrane potential.
3 Na⁺
Membrane potential = −50 to −70 mV
Na⁺K⁺ ATPase
Plasma membrane
ATP
ADP + Pᵢ
2 K⁺
(b)
Ions tend to move down their electrochemical gradient across the polarized membrane.
Low [Na⁺] High
High [K⁺] Low
Low [Ca²⁺] High
Low [Cl⁻] High
+
−

**Figure 12-24**
*Lehninger Principles of Biochemistry, Fifth Edition*
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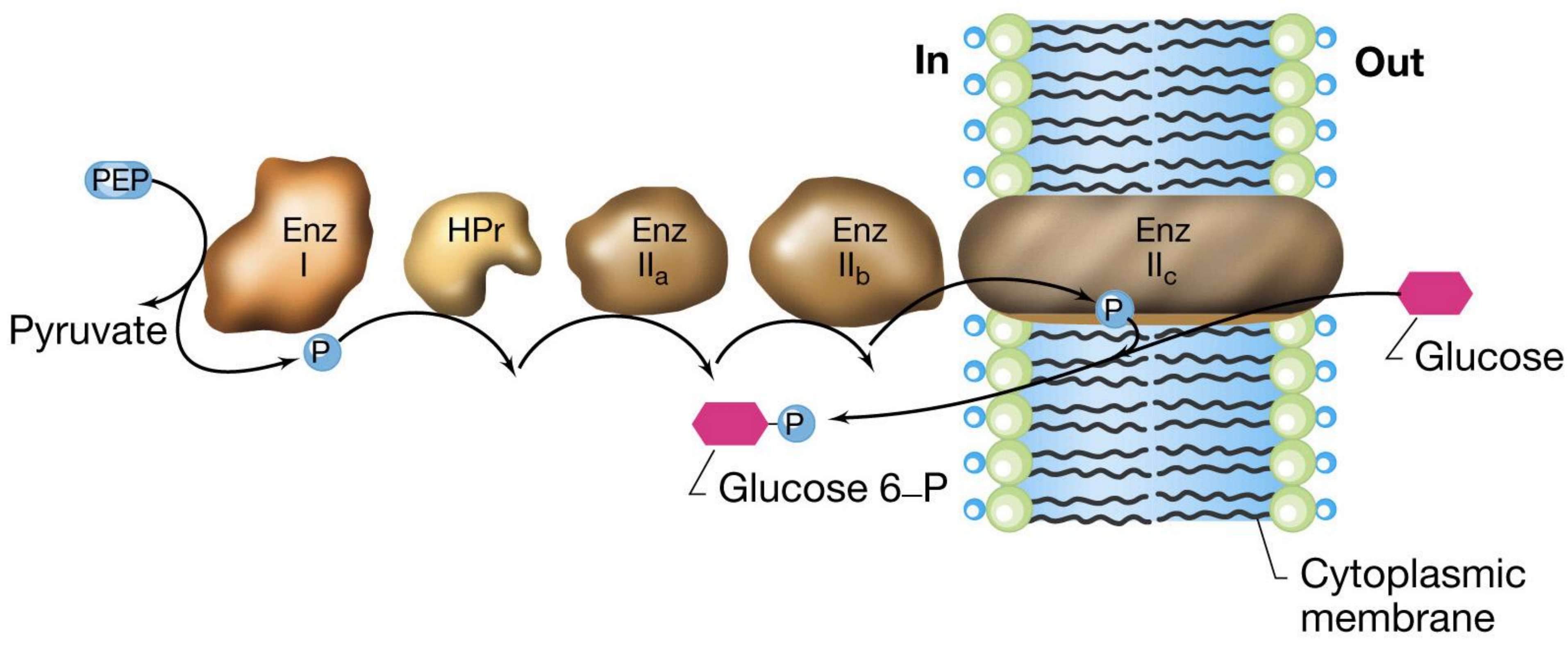
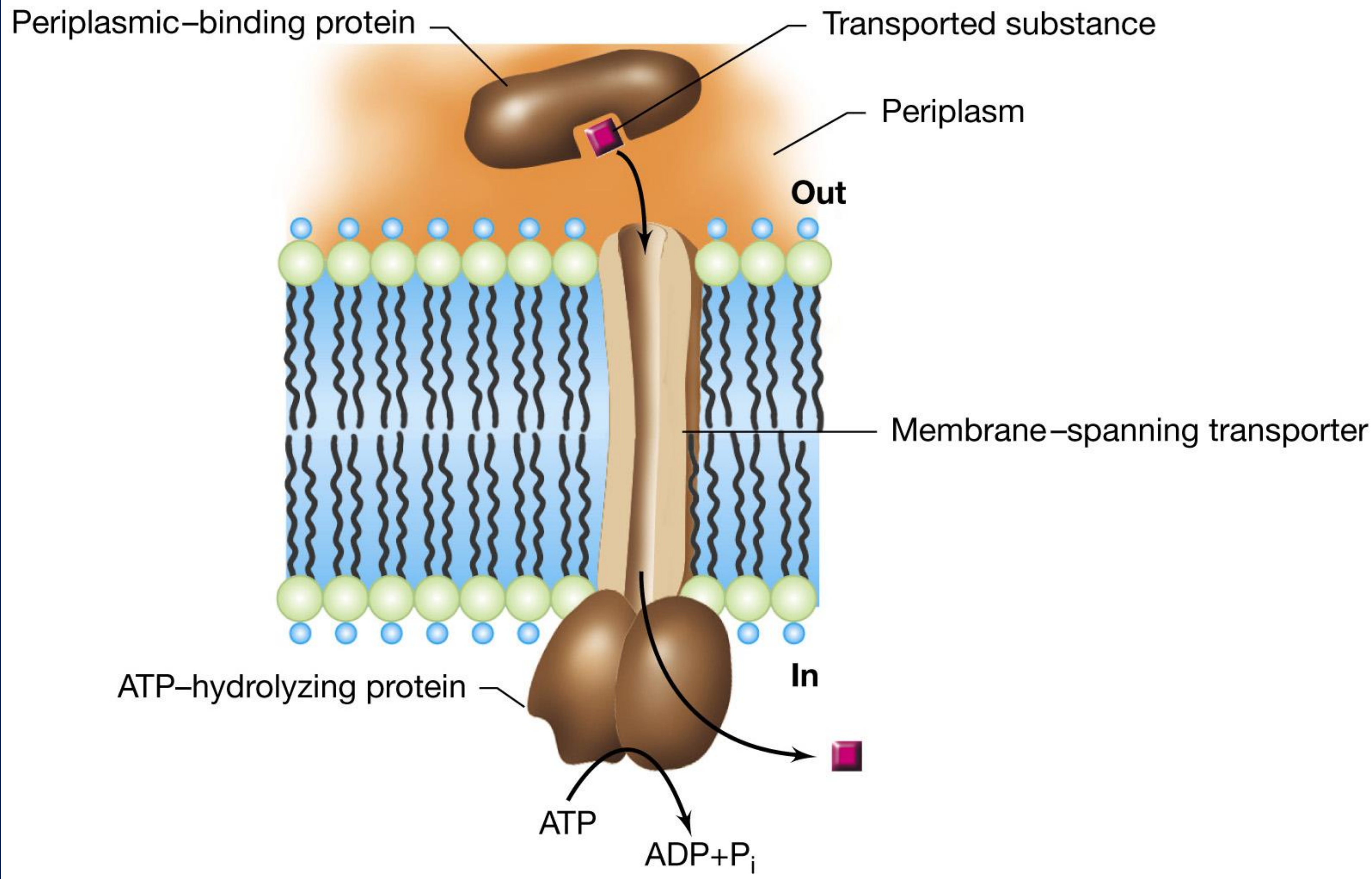
# Group translocation



# ATP-Binding Cassette (ABC-type) transporter



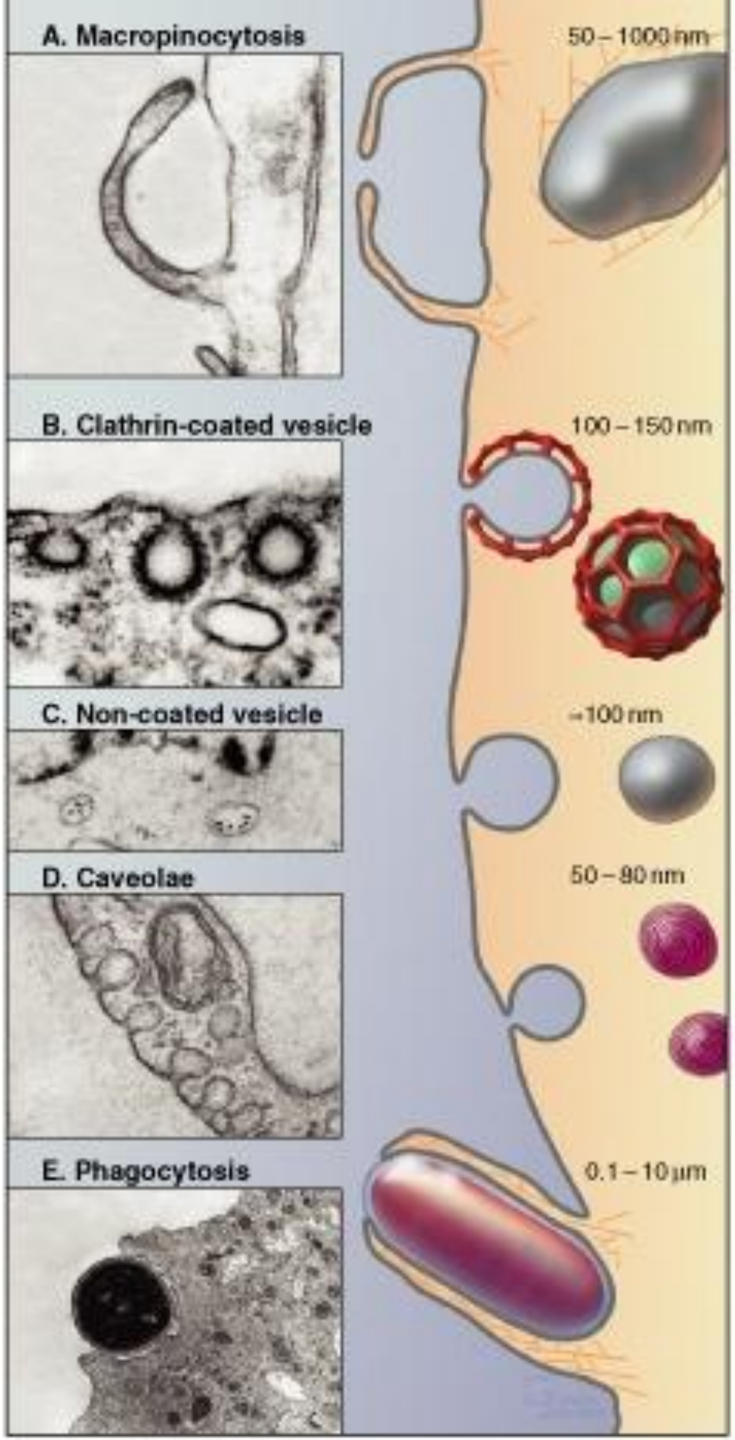
# Some of the functions of endocytosis

- Nutrient uptake
- Receptor recycling
- Plasma membrane protein downregulation and/or degradation
- Synaptic vesicle recycling
- Transcellular signaling
- Exploitation: virus and toxin entry into cells

# Common features of endocytosis

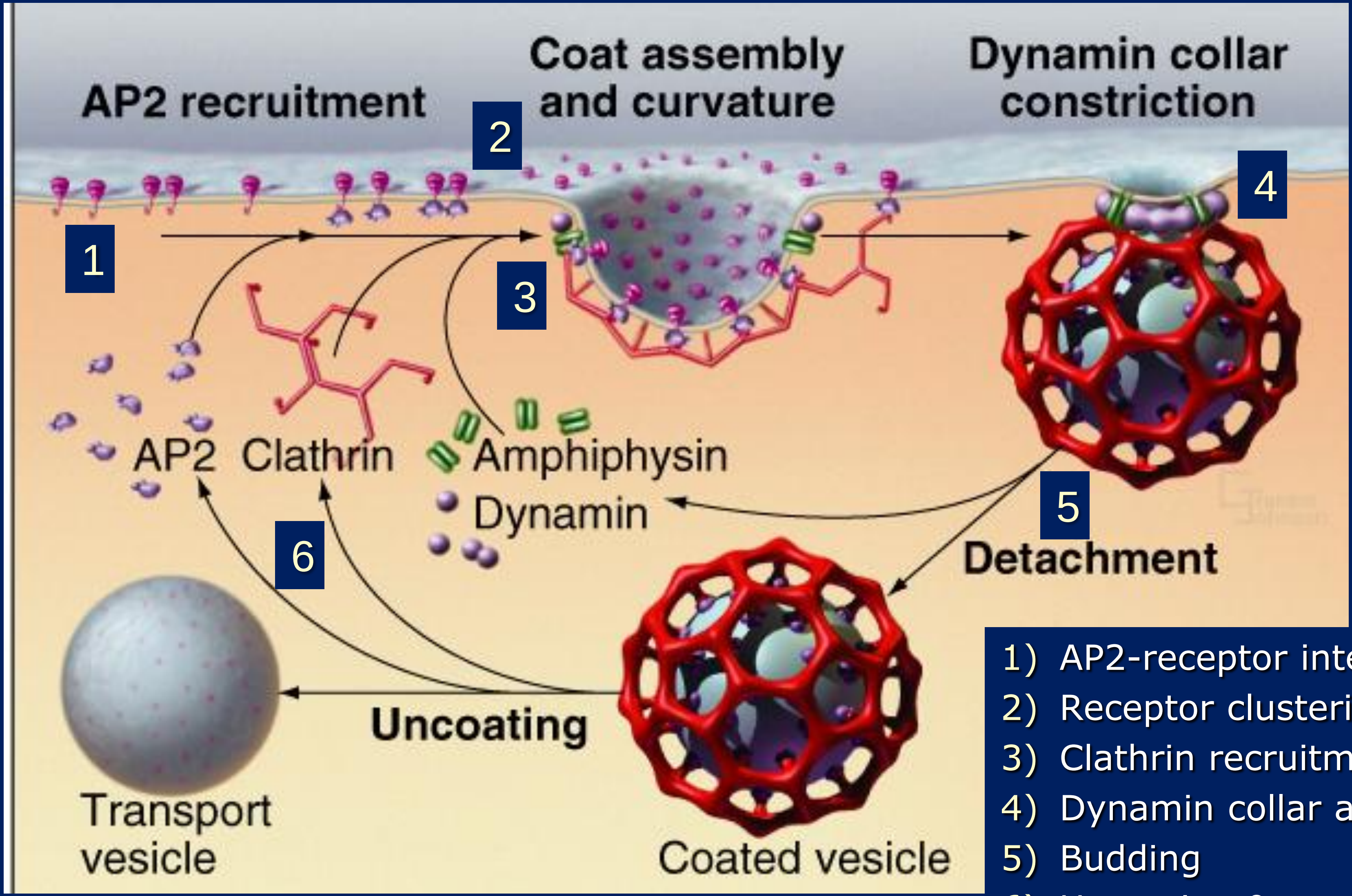
- Small region of the plasma membrane invaginates to form *membrane-limited vesicles* (0.05-0.1 mm diameter)
- Internalized molecules *retain topology* (lumenal = extracellular)
- Cargo can be *specifically selected*
- Destination of cargo can be *controlled*; destination depends on pathway of uptake
- Cargo *receptors can be recycled* to the cell surface
- Conditions under which internalization occurs can be regulated
- Cytoskeletal and coating proteins involvement

# Endocytic Mechanisms



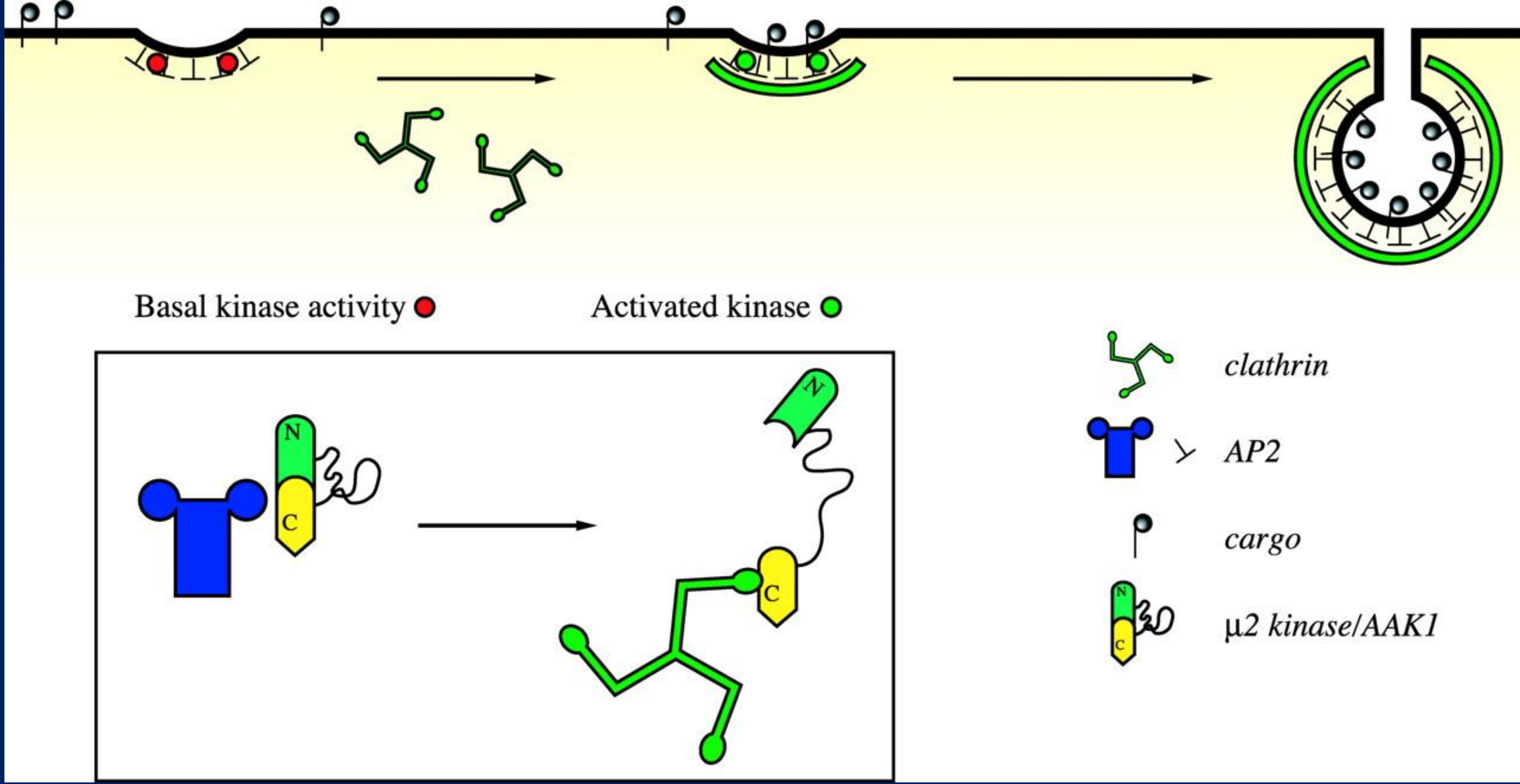
- clathrin coated pits
- caveolin coated caveolae
- non-coated pit pinocytosis
- cytoskeletal derived
  - macropinocytosis
  - phagocytosis

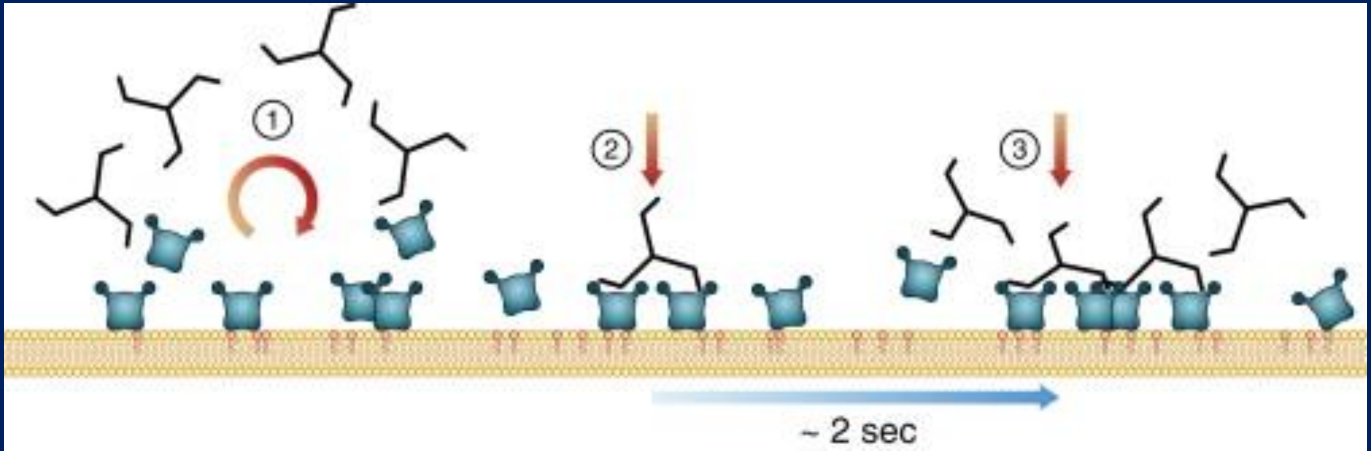
# Formation of Coated Pit

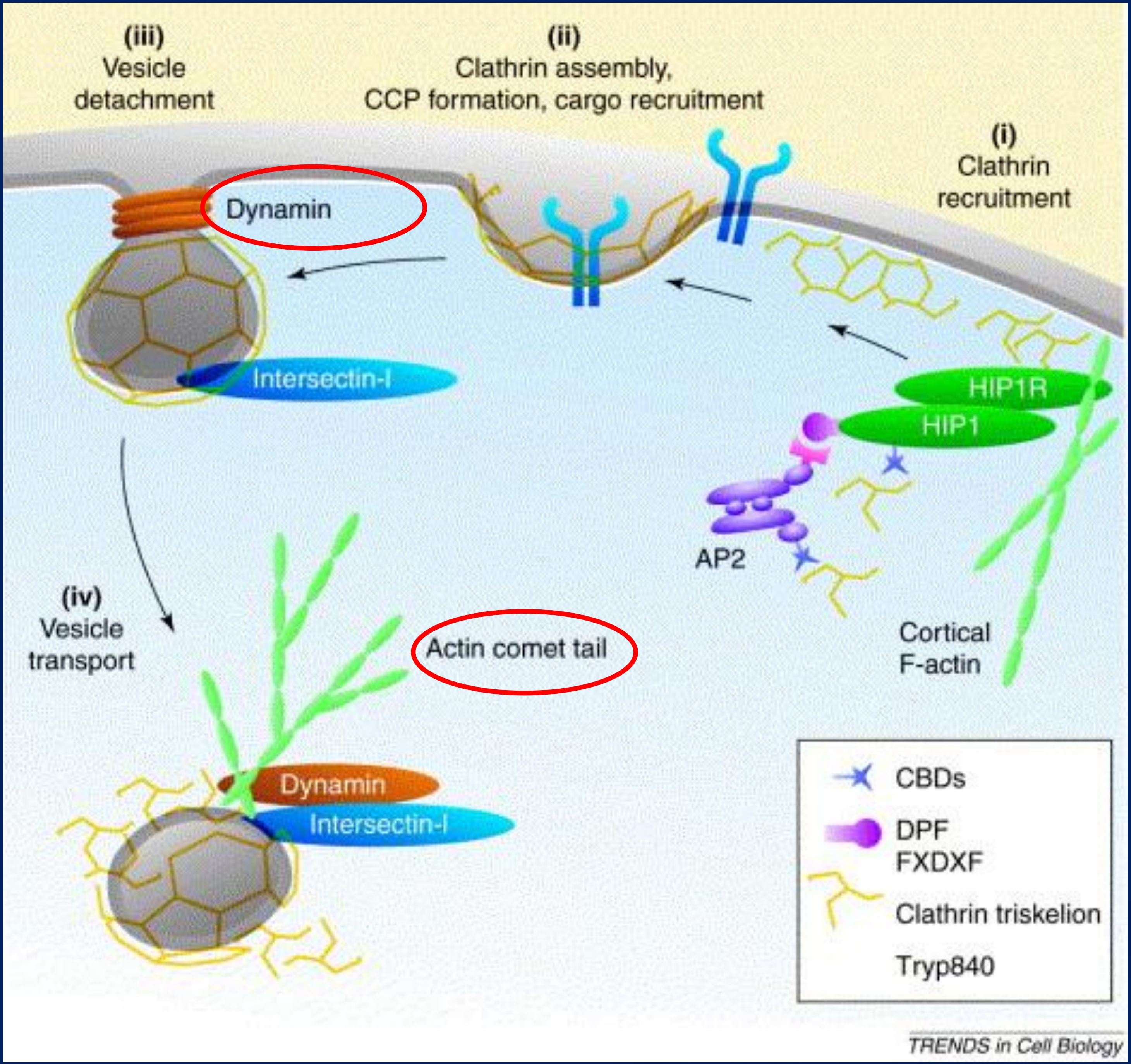


1) AP2-receptor interaction
2) Receptor clustering
3) Clathrin recruitment
4) Dynamin collar assembly
5) Budding
6) Uncoating & recycling of components

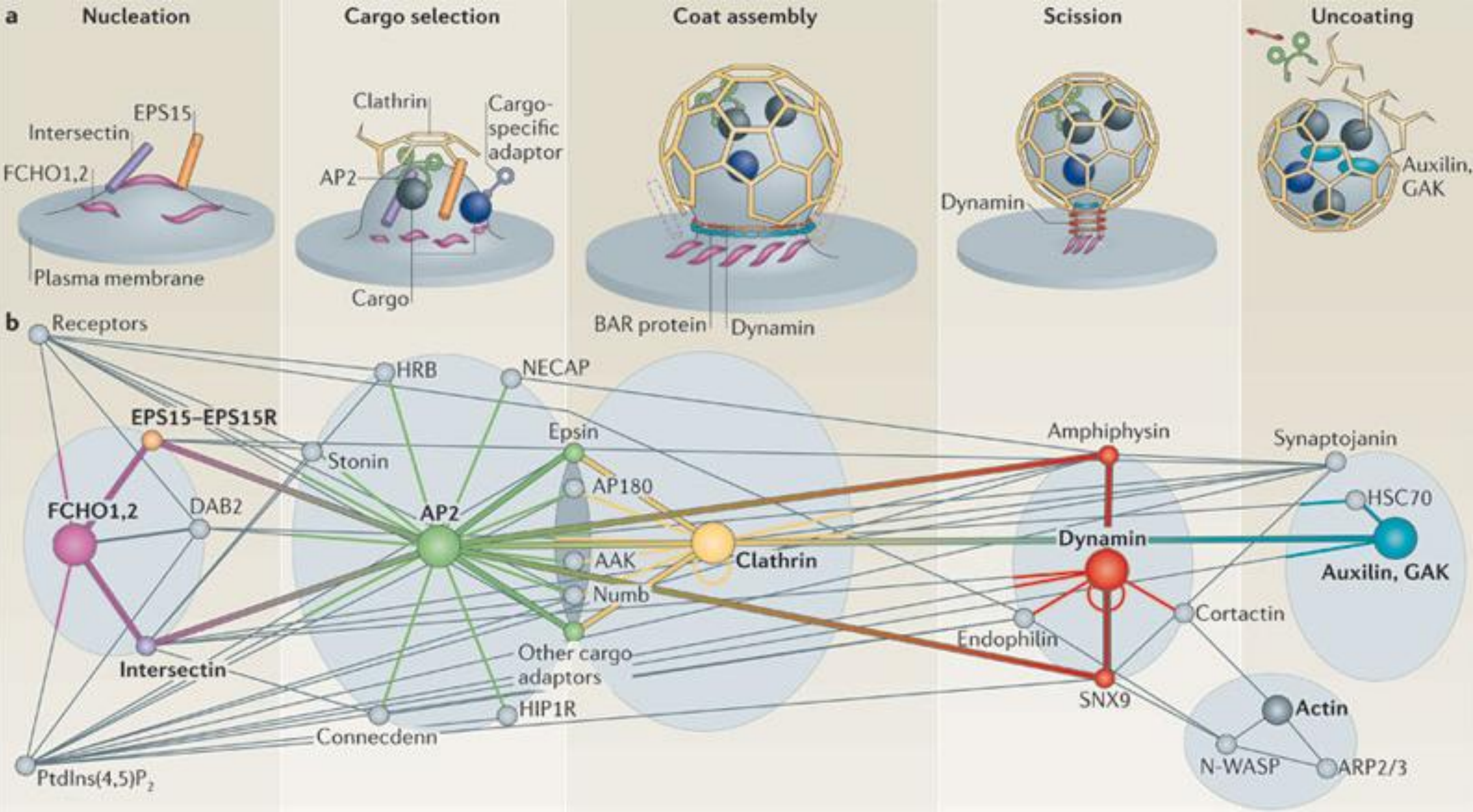
AP=accessory protein


Basal kinase activity ●
Activated kinase ●
N
C
N
C
clathrin
AP2
cargo
μ2 kinase/AAK1
N
C

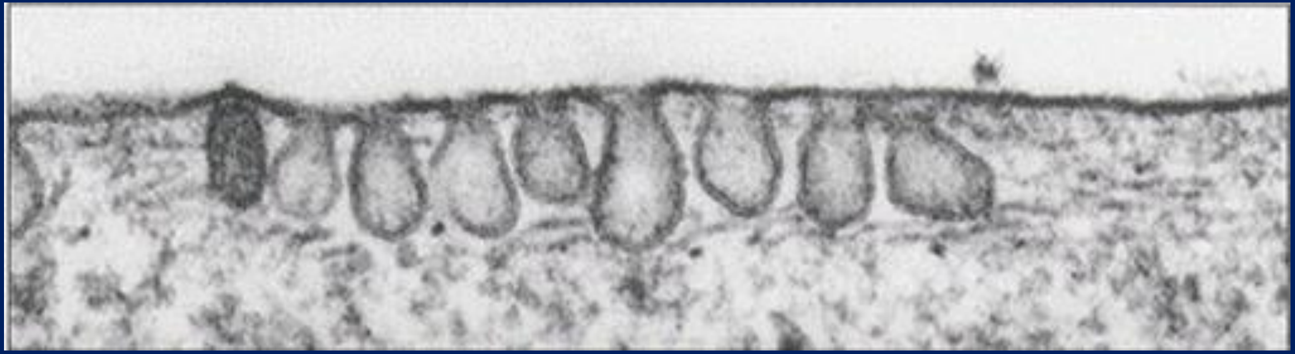

①
②
③
~ 2 sec



TRENDS in Cell Biology


a
Nucleation
Cargo selection
Coat assembly
Scission
Uncoating
EPS15
Intersectin
FCHO1,2
Plasma membrane
Clathrin
Cargo-specific adaptor
AP2
Cargo
BAR protein
Dynamin
Dynamin
Auxilin, GAK
b
Receptors
HRB
NECAP
EPS15–EPS15R
Stonin
Epsin
AP180
FCHO1,2
DAB2
AP2
Clathrin
AAK
Numb
Other cargo adaptors
Intersectin
Connecdenn
HIP1R
PtdIns(4,5)P₂
Amphiphysin
Synaptojanin
HSC70
Dynamin
Auxilin, GAK
Endophilin
Cortactin
SNX9
Actin
N-WASP
ARP2/3

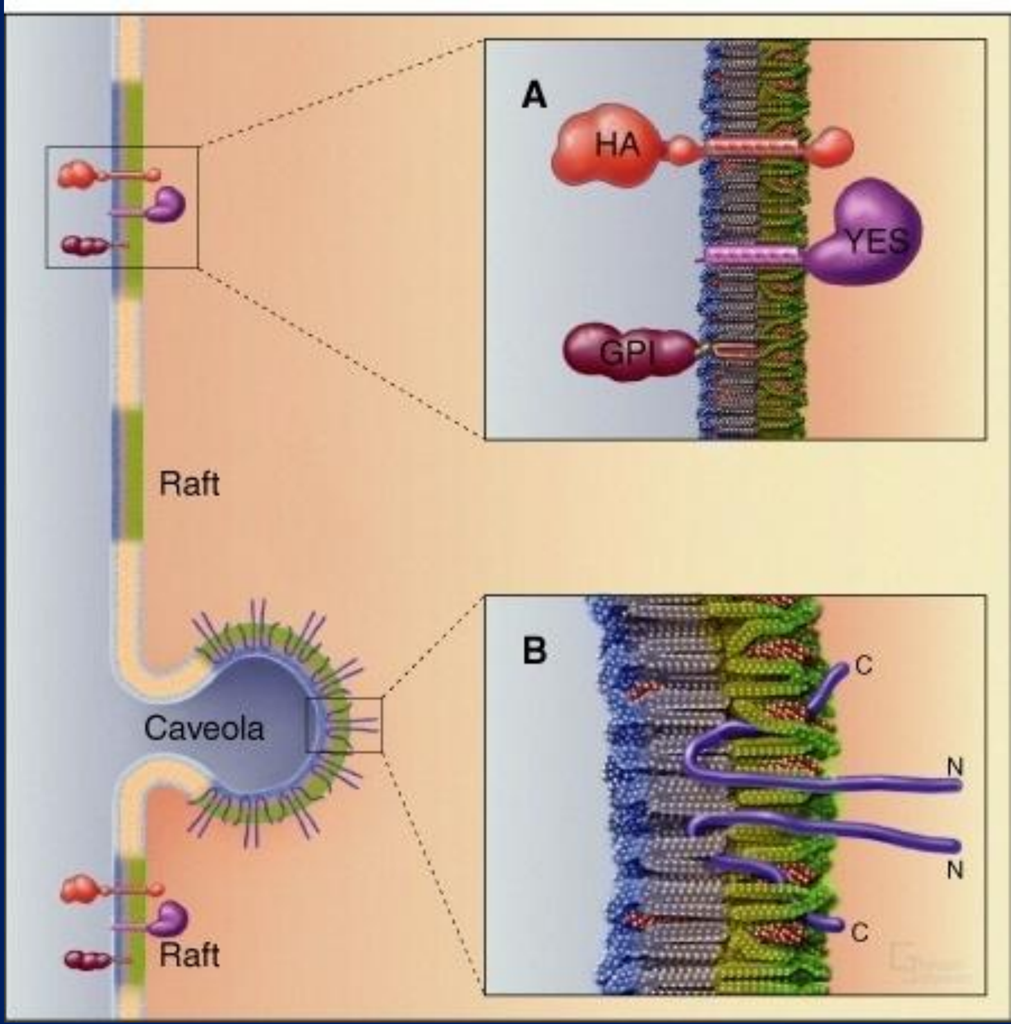
# Caveolae



- Caveolae are 50-100 nm invaginations on the cells surface
  - Caveolin, a membrane protein, is the “coat protein” of caveolae
- Do not undergo constitutive endocytosis but can undergo ***endocytosis in response to a signal*** (ex. SV40 binding) in a cholesterol- and dynamin-dependent fashion
- Internalized caveolae recruit actin to form “comet tails”
- Upon internalization caveolae are delivered to novel endosomal compartments known as “caveosomes”

# Caveolae

- Associated with lipid rafts
- Membrane lipid cycling
- **Raft-associated protein endocytosis/exocytosis**
  - Inner leaflet proteins
    - acylated proteins
    - prenylated proteins
  - Outer leaflet proteins
    - GPI-linked
  - Some TM proteins that interact specifically with glycosphingolipids/ cholesterol
- Caveolin
  - **high-affinity cholesterol binding protein**



# Clathrin viz caveolae endocytosis

- ☐ Both caveolae and clathrin-mediated endocytosis involves dynamin
- ☐ Caveolae endocytosis can be inhibited by dominant negative caveolin
- ☐ Caveolae endocytosis is inhibited by cholesterol depletion

- Can be Non-clathrin/non-caveolae (Currently poorly understood)

# Pinocytosis/ phagocytosis

- Pinocytosis: internalization of fluid
  - Actin dependent
  - Generated at sites of ruffling at the plasma membrane
  - Includes macropinocytosis (vesicles > 1 mm in diameter) and micropinocytosis (vesicles < 200nm in diameter)
- Phagocytosis: internalization of particles
  - Occurs in specialized cells ex. neutrophils and macrophages
  - Actin dependent
  - Clathrin independent
  - Particles > 0.5 mm in diameter

# Pretty Colored Vesicles

- Gold dust through the endolytic pathway
  - coated pits (CP)
  - early endosome (EE)
  - late endosome (LE)
  - lysosome (LY)